

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

THIS PAGE BLANK (USPTO)

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁶ : C12N 15/19, 15/12, C07K 14/52, 14/715, C12N 15/62, A61K 15/62, 38/16, C07K 16/24, 16/28, G01N 33/566, 33/577, 33/68</p>		<p>A2</p>	<p>(11) International Publication Number: WO 97/21812 (43) International Publication Date: 19 June 1997 (19.06.97)</p>
<p>(21) International Application Number: PCT/US96/19139 (22) International Filing Date: 5 December 1996 (05.12.96)</p>		<p>(81) Designated States: JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p>	
<p>(30) Priority Data: 08/567,882 8 December 1995 (08.12.95) US</p>		<p>Published <i>Without international search report and to be republished upon receipt of that report.</i></p>	
<p>(71) Applicant: SCHERING CORPORATION [US/US]; 2000 Galloping Hill Road, Kenilworth, NJ 07033-0530 (US).</p>			
<p>(72) Inventors: DAIRAGHI, Daniel, J.; 2080 Cornell Street, Palo Alto, CA 94306 (US). HARA, Takahiko; 3-4-3-201, Ogorodai, Inzaimachi, Inagun Chiba 270-13 (JP). MIYAJIMA, Atsushi; 1-3-17-811, Ecchujima, Kotoh-ku, Tokyo 135 (JP). SCHALL, Thomas, J.; 2050 Mills Avenue, Menlo Park, CA 94025 (US). WANG, Wei; 3090 Middlefield Road, Palo Alto, CA 94036 (US). YOSHIMURA, Akihiko; 2526-12, Miimachi, Kurume-shi (JP).</p>			
<p>(74) Agents: FOULKE, Cynthia, L. et al.; Schering-Plough Corporation, Patent Dept. K-6-1 1990, 2000 Galloping Hill Road, Kenilworth, NJ 07033-0530 (US).</p>			
<p>(54) Title: MAMMALIAN CHEMOKINE CCP8 AND CHEMOKINE RECEPTOR CCKR3</p>			
<p>(57) Abstract</p>			
<p>A novel CC-chemokine which is from a mammal, reagents related thereto including purified proteins, specific antibodies, and nucleic acids encoding said chemokine. A chemokine receptor is also provided. Methods of using said reagents and diagnostic kits are also provided.</p>			

AML INFORMATION SERVICES
P.O. BOX 405, CORTE MADERA, CA 94976-0405
(415) 927-0340 FAX (415) 927-7250

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CP	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

MAMMALIAN CHEMOKINE CCF8 AND CHEMOKINE RECEPTOR CCKR3

FIELD OF THE INVENTION

5 The present invention relates to compositions related to proteins which function in controlling development and differentiation of mammalian cells, e.g., cells of a mammalian immune system. In particular, it provides proteins and mimetics which regulate development, differentiation, and
10 function of various cell types, including hematopoietic cells. It also provides receptor reagents for such proteins.

BACKGROUND OF THE INVENTION

15 The circulating component of the mammalian circulatory system comprises various cell types, including red and white blood cells of the erythroid or the myeloid cell lineages. See, e.g., Rapaport (1987) Introduction to Hematology (2d ed.) Lippincott, Philadelphia, PA; Jandl (1987) Blood: Textbook of Hematology, Little, Brown and Co., Boston, MA.;
20 and Paul (ed.) (1993) Fundamental Immunology 3d ed, Raven Press, N.Y. Progression through various stages of differentiation are regulated by various signals provided to the cells, often mediated through a class of proteins known as the cytokines. Within this group of molecules are a
25 further group known as the chemoattractant cytokines, or chemokines. See, e.g., Schall (1994) "The Chemokines" in The Cytokine Handbook (2d ed.) Academic Press; Schall and Bacon (1994) Current Opinion in Immunology 6:865.

30 Although the full spectrum of biological activities of the chemokines has not been extensively investigated, chemoattractant effects are recognized. The best known biological functions of these molecules relate to chemoattraction of leukocytes. However, new chemokines are being discovered, and their biological effects on the various
35 cells responsible for immunological responses are topics of continued study. Mouse CCF18 has been reported by a Japanese Group, and also published by the present inventors in Hara, et al. (1995) J. Immunol. 155:5352.

2

These observations indicate that other factors exist whose functions in hematopoiesis, immune development, and leukocyte trafficking were heretofore unrecognized. These factors provide for biological activities whose spectra of effects are distinct from known differentiation, activation, or other signaling factors. The absence of knowledge about the structural, biological, and physiological properties of the regulatory factors which regulate hematopoietic cell physiology in vivo prevents the modification of the effects of such factors. Thus, medical conditions where regulation of the development or physiology of relevant cells is required remain unmanageable.

SUMMARY OF THE INVENTION

15 The present invention is based, in part, upon the discovery of new genes encoding CC chemokines, and new genes encoding various receptors for chemokines. It embraces agonists and antagonists of the chemokine designated CCF18, e.g., mutations (muteins) of the natural sequences, fusion 20 proteins, chemical mimetics, antibodies, and other structural or functional analogs. It is also directed to isolated genes encoding proteins of the invention. Various uses of these different protein or nucleic acid compositions are also provided. Likewise for the receptor described herein.

25 The present invention provides a substantially pure CCF18 chemokine; a fusion protein comprising CCF18 chemokine sequence; an antibody specific for binding to a CCF18 chemokine; and a nucleic acid encoding a CCF18 chemokine or fusion protein thereof.

30 In CCF18 chemokine embodiments, the chemokine may be from a warm blooded animal selected from the group of birds and mammals, including a mouse or human; may comprise a sequence of Table 1 or 3; may exhibit a post-translational modification pattern distinct from natural CCF18 chemokine; 35 or may exhibit the features disclosed in Table 2. The invention also embraces a composition comprising the chemokine and a pharmaceutically acceptable carrier.

In fusion protein embodiments, the protein may comprise either sequence of Table 1 or 3; and/or sequence of another cytokine or chemokine.

5 In antibody embodiments, the CCF18 chemokine can be a mammalian protein, including a mouse or human; or the antibody may be raised against a peptide sequence of Table 1 or 3; may be monoclonal antibody; or the antibody may be labeled.

10 In nucleic acid embodiments, the chemokine may be from a warm blooded animal selected from the group of birds and mammals, including a mouse or human. The nucleic acid may comprise a sequence of Table 1 or 3; be an expression vector; or comprise a deoxyribonucleic acid nucleotide.

15 The invention also provides a kit comprising a substantially pure CCF18 chemokine, or fragment thereof; an antibody which specifically binds a mammalian CCF18 chemokine; or a nucleic acid encoding a CCF18 chemokine or peptide. The kit may also be capable of making a qualitative or quantitative analysis.

20 In another embodiment, the invention provides methods of modulating physiology or development of a cell comprising contacting the cell with an agonist or antagonist of a mammalian CCF18 chemokine. The antagonist may be an antibody against a mammalian CCF18 chemokine. The cell may be a 25 hematopoietic cell, including a lymphoid cell; a placenta cell; a gonad cell; or a neural cell, including neuronal or non-neuronal cells. Various of the physiological effects include effecting a cellular calcium flux; a chemoattractant response; cellular morphology modification responses; 30 phosphoinositide lipid turnover; or an antiviral response.

The present invention also provides reagents related to a chemokine receptor, designated CCKR3. In particular embodiments, the invention provides a substantially pure CCKR3 chemokine receptor; a fusion protein comprising CCKR3 chemokine receptor sequence; an antibody specific for binding to a CCKR3 chemokine receptor; and a nucleic acid encoding a CCKR3 chemokine receptor or fusion protein thereof.

5 The CCKR3 may be from a warm blooded animal selected from the group of birds and mammals, including a mouse or human; may comprise a sequence of Table 4; or may exhibit a post-translational modification pattern distinct from natural CCKR3 chemokine receptor.

In receptor fusion embodiments, the protein may comprise sequence of Table 4; and/or sequence of another receptor for a chemokine.

10 In receptor antibody embodiments, the CCKR3 chemokine receptor may be a mammalian protein, including a mouse or human. The antibody may be raised against a peptide sequence of Table 4; may be a monoclonal antibody; or may be labeled.

15 In receptor nucleic acid embodiments, the chemokine receptor may be from a warm blooded animal selected from the group of birds and mammals, including a mouse or human. Alternatively, the nucleic acid may comprise a sequence of Table 4; may be an expression vector; or may comprise a deoxyribonucleic acid nucleotide.

20 DETAILED DESCRIPTION OF THE INVENTION

25 The present invention provides DNA sequences encoding various mammalian proteins which exhibit structural properties characteristic of a chemotactic cytokine, or chemokine. See, e.g., Lodi, et al. (1994) Science 263:1762; Gronenborn and Clore (1991) Protein Engineering 4:263; Miller and Kranger (1992) Proc. Nat'l Acad. Sci. USA 89:2950; Matsushima and Oppenheim (1989) Cytokine 1:2; Stoeckle and Baker (1990) New Biol. 2:313; Oppenheim, et al. (1991) Ann. Rev. Immunol. 9:617; Schall (1991) Cytokine 3:165; and The Cytokine Handbook Academic Press, NY. Both mouse and human embodiments are described herein.

30 35 Chemokines play an important role in immune and inflammatory responses by inducing migration and adhesion of leukocytes. These small secreted molecules are a growing superfamily of 8-14 kDa proteins characterized by a conserved four cysteine motif. See, e.g., Schall (1991) Cytokine 3:165; and The Cytokine Handbook Academic Press, NY.

Chemokines are secreted by activated leukocytes and act as a chemoattractant for a variety of cells which are involved in inflammation. Besides chemoattractant properties, chemokines have been shown to induce other biological responses, e.g.,

5 modulation of second messenger levels such as Ca^{++} ; inositol phosphate pool changes (see, e.g., Berridge (1993) Nature 361:315 or Billah and Anthes (1990) Biochem. J. 269:281); cellular morphology modification responses; phosphoinositide lipid turnover; possible antiviral responses; and others.

10 Thus, CCF18 may, alone or in combination with other therapeutic reagents, have advantageous combination effects. There are reasons to suggest that chemokines may have effects on other cell types, e.g., attraction or activation of monocytes, dendritic cells, T cells, eosinophils, and/or

15 perhaps on basophils and/or neutrophils. They may also have chemoattractive effects on various neural cells including, e.g., dorsal root ganglia neurons in the peripheral nervous system and/or central nervous system neurons.

The chemokine superfamily is mainly divided into two

20 main groups exhibiting characteristic structural motifs, the Cys-X-Cys (C-X-C) and Cys-Cys (C-C) families. These are distinguished on the basis of a single amino acid insertion between the NH-proximal pair of cysteine residues and sequence similarity. Typically, the C-X-C chemokines, i.e.,

25 IL-8 and MGSA/Gro- α act on neutrophils but not on monocytes, whereas the C-C chemokines, i.e., MIP-1 α and RANTES, are potent chemoattractants for monocytes and lymphocytes but not neutrophils. See, e.g., Miller, et al. (1992) Crit. Rev. Immunol. 12:17. A recently isolated chemokine, lymphotactin,

30 does not belong to either group and may constitute a first member of a third chemokine family, the C family. Lymphotactin does not have a characteristic CC or CXC motif, and acts on lymphocytes but not neutrophils and monocytes. See, e.g., Kelner, et al. (1994) Science 266:1395. The

35 chemokine molecule described herein is a member of the C-C chemokine family and is designated CCF18.

In another embodiment, the invention provides a gene encoding a chemokine receptor designated CC chemokine

receptor 3, or CCKR3. Its ligand has not yet specifically been identified. However, the receptor exhibits structural features typical of known chemokine receptors, e.g., it is a 7 transmembrane spanning structure. It may exhibit 5 properties of binding many different cytokines at varying specificities (shared or promiscuous specificity) or may exhibit high affinity for one (specific) or a subset (shared) of the chemokines.

10 The described chemokine and receptor should be important for mediating various aspects of cellular physiology or development.

Purified CCF18 chemokine

15 Mouse CCF18 chemokine nucleotide and amino acid sequences are shown in Table 1. Nucleotide and amino acid sequences of a human chemokine are provided in Table 3. The mouse and human nucleotide sequences correspond to SEQ ID NO: 1 and 3, respectively, and the mouse and human amino acid sequences correspond to SEQ ID NO: 2 and 4, respectively. 20 The human chemokine exhibits similarity at the protein level of about 50% to the mouse counterpart. The nucleotide and amino acid sequences of the chemokine receptor, CCKR3, are provided in Table 4 and SEQ ID NO: 6 and 7. These amino acid sequences, provided amino to carboxy, are important in 25 providing sequence information on the ligand allowing for distinguishing the protein from other proteins. Moreover, the peptide sequences allow preparation of peptides to generate antibodies to recognize such segments, and allow preparation of oligonucleotide probes, both of which are 30 strategies for isolation, e.g., cloning, of genes encoding such sequences. Similarities have been observed with other cytokines. See, e.g., Bosenberg, et al. (1992) Cell 71:1157; Huang, et. al. (1992) Molecular Biology of the Cell 3:349; and Pandiella, et al. (1992) J. Biol. Chem. 267:24028. This 35 will avoid certain problems of working with, or administering, a cell bound protein, and provides insight into possible mechanisms of cellular specificity.

TABLE 1

5 Nucleotide sequence (5' to 3') of CCF18 chemokine from mouse and the corresponding amino acid sequence (amino to carboxy), SEQ ID NO: 1 and 2. The conserved four cysteine residues and one potential RNA destabilizing sequence (ATTTA) are indicated by asterisks underneath. A predicted signal sequence, one potential N-glycosylation site (Asn-X-Ser/Thr), and two poly(A) addition signals (AATAAA) are underlined.

10

15

20

25

30

35

40

45

50

55

60

GCCTCGTGCC	GAATTGGCA	CGAGGGCCA	GCTGGTCTG	CCCACTAAGA	AG	52
ATG AAG CCT TTT CAT ACT GCC CTC TCC TTC CTC ATT CTT ACA ACT GCT	Met Lys Pro Phe His Thr Ala Leu Ser Phe Leu Ile Leu Thr Thr Ala				100	
					16	
CTT GGA ATC TGG GCC CAG ATC ACA CAT GCA ACA GAG ACA AAA GAA GTC	Leu Gly Ile Trp Ala Gln Ile Thr His Ala Thr Glu Thr Lys Glu Val				148	
CAG AGC AGT CTG AAG GCA CAG CAA GGG CTT GAA ATT GAA ATG TTT CAC	Gln Ser Ser Leu Lys Ala Gln Gly Leu Glu Ile Glu Met Phe His				196	
Met Gly Phe Gln Asp Ser Ser Asp Cys Cys Leu Ser Tyr Asn Ser Arg					48	
ATG GGC TTT CAA GAC TCT TCA GAT TGC TGC CTG TCC TAT AAC TCA CGG					244	
Met Gly Phe Gln Asp Ser Ser Asp Cys Cys Leu Ser Tyr Asn Ser Arg					64	
ATT CAG TGT TCA AGA TTT ATA GGT TAT TTT CCC ACC AGT GGT GGG TGT					292	
Ile Gln Cys Ser Arg Phe Ile Gly Tyr Phe Pro Thr Ser Gly Gly Cys					80	
ACC AGG CCG GGC ATC ATC TTT ATC AGC AAG AGG GGG TTC CAG GTC TGT					340	
Thr Arg Pro Gly Ile Ile Phe Ile Ser Lys Arg Gly Phe Gln Val Cys					96	
GCC AAC CCC AGT GAT CGG AGA GTT CAG AGA TGC ATT GAA AGA TTG GAG					388	
Ala Asn Pro Ser Asp Arg Arg Val Gln Arg Cys Ile Glu Arg Leu Glu					112	
CAA AAC TCA CAA CCA CGG ACC TAC AAA CAA TAA CATTGCTTG AAGAGAAGGG					441	
Gln Asn Ser Gln Pro Arg Thr Tyr Lys Gln end					122	
TGTGAACGTGC CAGCTACTTT CTTTGGTCTT CCCCAGTGAC CACCTAAGCG GCTCTAAGTG					501	
TTTATTTTTA TAGGTATATA AACATTTTTT TTCTGTTCC ACTTTAAAGT GGCATATCTG					561	
GCTTGTACAC AGAGGGAAA CTTGTCTGTG CCAACCCAG TCATCTGAAA ACTCAGATGC					621	
CTGCGGAAGG TCTGAAGCTG ACTCAATGAC TACACATAAT ATTTGATTGA GATAAATGGG					681	
CAAGGTCTGG AGAGATGGCT TGGTGGTTAA GAGCACCTGC TGCTCTTCCA GAGGACCTGG					741	
GTTCAATTCC CACTTAGATG GCAGCTAAA CTATCTATAA TTCCAATTCC AAAGAAAAT					801	
GATGCCCTAT TTTGCCCTTT AGTTAGTAGT ATTTACAGTA TTCTTTATAA ATTCACTTGTG					861	

ACATGACCAT CTTGAGCTAC AGCCATCCTA ACTGCCTCAG AATCACTCAA GTTCTTCCAC					921	
TCGGTTTCCC AGCGGATTAT AAGTGGATAA ACTGTGAGAG TGGTCTGTGG GACTTTGGAA					981	
TGTGTCTGGT TCTGATAGTC ACTTATGGCA ACCCGGGTAC ATTCAACTAG GATGAAATAA					1041	
ATTCTGCCTT AGCCCAGTAG TATGTCTGTG TTTGTAAGGA CCCAGCTGAT TTTCCCACCA					1101	

8

CCCCCTCCATC AGTAAGCCAC TAATAAAAGTG CATCTATGCA GCCAAAAAAA AAAAAA 1161

AAAAA

1065

5

TABLE 2

Physical properties of mouse CCF18 chemokine.

10 - open reading frame encoding a polypeptide of about 123 amino acids
- mature protein of about 102 amino acids
- 4 cysteine residues
- molecular weight of about 11549 daltons
- one potential N-glycosylation site

15

TABLE 3

Human CC chemokine nucleic acid coding sequence.
SEQ ID NO: 3 and 4.

1	ATGAAAGGTCTCCGTGGCTGCCCTCTGCCATGCTTGTGCTGCTGGATCCCAG	60
1	MetLysValSerValAlaAlaLeuSerCysLeuMetLeuValAlaValLeuGlySerGln	20
25	61 GCCCAGTTCATAAATGATGCAGAGACAGAGTTAATGATGTCAAAGCTTCCACTGGAAAAT	120
	21 AlaGlnPheIleAsnAspAlaGluThrGluLeuMetMetSerLysLeuProLeuGluAsn	40
30	121 CCAGTAGTTCTGAACAGCTTCACTTTGCTGACTGCTGCACCTCCTACATCTCACAA	180
	41 ProValValLeuAsnSerPheHisPheAlaAlaAspCysCysThrSerTyrIleSerGln	60
	181 AGCATCCCGTGTTCACTCATGAAAAGTTATTTGAAACGAGCAGCGAGTGCTCCAAGCCA	240
	61 SerIleProCysSerLeuMetLysSerTyrPheGluThrSerGluCysSerLysPro	80
35	241 GGTGTCATATTCCCTCACCAAGAAGGGGCGCAAGTCTGCCAAACCCAGTGGTCCGGGA	300
	81 GlyValIlePheLeuThrLysLysGlyArgGlnValCysAlaLysProSerGlyProGly	100
	301 GTTCAGGATTGCATGAAAAAGCTGAAGCCCTACTCAATATAATAATAA 348	
	101 ValGlnAspCysMetLysLysLeuLysProTyrSerIleEndEndEnd 116	

40 Full length human CC chemokine

CACTGAGGCCAGGAGTCCTCGGCCAGCCCTGCCTGCCACCAGGAGG
ATGAAGGTCTCCGTGGCTGCCCTCCTGCCATGCTGTTGCTGCCCTGGATCCCAGG
CCCAGTTCATAAATGATGCAGAGACAGAGTTAATGATGTCAAAGCTCCACTGGAAAATCC
AGTAGTTCTGAACAGCTTCACTTTGCTGCTGACTGCTGCACCTCCTACATCTCACAAAGC
ATCCCCGTGTTCACTCATGAAAAGTTATTTGAAACGAGCAGCGAGTGCTCCAAGCCAGGTG
TCATATTCTCACCAGAAGGGGGNGCCAAGTCTGCTGCCAAACCCAGTGGTCCGGGAGTTCA
GGATTGCATGAAAAAGCTGAAGCCCTACTCAATATAATAAAAGAGACAAAAGAGGNAG
CCACCCACCTCAACACCTCTGTGAGTTCTTGGCTGAAATACTTAAAAAATATATA
TTGTTGTGCTGGTAATGAAAGTAATGCATCTAATAAAGAGTATTCAATTTTAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAANCTCGGGGGGGGGCCGGTCCCCAATCCG
CCCTNTGGGGAGTCGTTNAAAATCCACTGGCCGCCGTTTAAAACGTNGGGATTGGGAAA
ACCCNGGGGTNCCAACTTANTCNCCTNGGAGAANATCCCCCTTCCGCCAGTTGGGGTT
AATAGGGAAGGAGGCCGNACCGATCCGCCCTCCCCAAAAGGNGGGAGN

TABLE 4

Human chemokine receptor, CCKR3, nucleotide and
amino acid sequences.
SEQ ID NO: 5 and 6.

5	1	ATGACAAACCTCACTAGATAACAGTTGAGACCTTGGTACCAACATCCTACTATGATGACGTG	60
	1	MetThrThrSerLeuAspThrValGluThrPheGlyThrThrSerTyrTyrAspAspVal	20
10	61	GGCCTGCTCTGTGAAAAAGCTGATAACCAAGAGCACTGATGGCCAGTTGTGCCCGCTG	120
	21	GlyLeuLeuCysGluLysAlaAspThrArgAlaLeuMetAlaGlnPheValProProLeu	40
	121	TACTCCCTGGTGTCACTGTGGCCCTTGGGCAATGTGGTGGTGGTGTGATGATCCTCATA	180
15	41	TyrSerLeuValPheThrValGlyLeuLeuGlyAsnValValValValMetIleLeuIle	60
	181	AAATACAGGAGGCTCCGAATTATGACCAACATCTACCTGCTCAACCTGGCCATTTGGAC	240
	61	LysTyrArgArgLeuArgIleMetThrAsnIleTyrLeuLeuAsnLeuAlaIleSerAsp	80
20	241	CTGCTCTCCTCGTCACCCTCCATTCTGGATCCACTATGTCAGGGGCATAACTGGGTT	300
	81	LeuLeuPheLeuValThrLeuProPheTrpIleHisTyrValArgGlyHisAsnTrpVal	100
	301	TTTGGGCCATGGCATGTGTAAGCTCCTCTCAGGGTTTTATCACACAGGCTTGTACAGCGAG	360
	101	PheGlyHisGlyMetCysLysLeuLeuSerGlyPheTyrHisThrGlyLeuTyrSerGlu	120
25	361	ATCTTTTCATAATCCTGCTGACAATCGACAGGTACCTGGCCATTGTCATGCTGTGTTT	420
	121	IlePhePheIleIleLeuLeuThrIleAspArgTyrLeuAlaIleValHisAlaValPhe	100
	421	GCCCTTCGAGCCCCACTGTCACCTTGTTGTTCATCACCAGCATCGTCACCTGGGCCTG	480
30	141	AlaLeuArgAlaArgThrValThrPheGlyValIleThrSerIleValThrTrpGlyLeu	160
	481	GCAGTGCTAGCAGCTTCTCGAACCTTATCTTCTATGAGACTGAAGAGTTGTTGAAGAG	540
	161	AlaValLeuAlaAlaLeuProGluPheIlePheTyrGluThrGluGluLeuPheGluGlu	180
	541	ACTCTTGCAGTCCTTACCCAGAGGATACAGTATATAGCTGGAGGCATTCCACACT	600
35	181	ThrLeuCysSerAlaLeuTyrProGluAspThrValTyrSerTrpArgHisPheHisThr	200
	601	CTGAGAACATGCCATCTCTGTCGTTCTCCCTCTGCTCGTATGGCCATCTGCTACACA	660
	201	LeuArgMetThrIlePheCysLeuValLeuProLeuLeuValMetAlaIleCysTyrThr	220
40	661	GGAATCATAAAAGCTGCTGAGGTGCCAGTAAAAAAAGTACAAGGCCATCCGGCTC	720
	221	GlyIleIleLysThrLeuLeuArgCysProSerLysLysTyrLysAlaIleArgLeu	240
	721	ATTTTTGTCATCATGGCGGTGTTTCATTCTGGACACCCTACAAATGTCGCTATCCTT	780
45	241	IlePheValIleMetAlaValPhePheIlePheTrpThrProTyrAsnValAlaIleLeu	260
	781	CTCTCTCCTATCAATCCATCTTATGGAAATGACTGTGAGCGGAGCAAGCATCTGGAC	840
	261	LeuSerSerTyrGlnSerIleLeuPheGlyAsnAspCysGluArgSerLysHisLeuAsp	280
	841	CTGGACATGCTGGACAGAGGTGATGCCACTCCCACGGTGCCTCAATCCCTC	900
50	281	LeuAspMetLeuValThrGluValIleAlaTyrSerHisTrpCysCysLeuAsnProLeu	300
	901	ATCTACGCCCTTGTGGAGAGAGGGTCCGGAAGTACCTGCGCCACTTCTCCACAGGCAC	960
	301	IleTyrAlaPheValGlyGluArgPheArgLysTyrLeuArgHisPhePheHisArgHis	320
55	961	TTGCTCATGCACCTGGCAGATACATCCCATTCCCTCCTAGTGAGAGCTGGAAAGAAC	1020
	321	LeuLeuMetHisLeuGlyArgTyrIleProPheLeuProSerGluLysLeuGluArgThr	340
	1021	AGCTCTGTCCTCCATCCACAGGGAGGCCAACTCTCTATTGTGTTTAG	1072
	341	SerSerValSerProSerThrGlyGluProGluLeuSerIleValPheStop	358

As used herein, the term "CCF18 chemokine" shall encompass, when used in a protein context, a protein having mouse amino acid sequences shown in Table 1, or human amino acid sequences shown in Table 3. The invention also embraces a polypeptide comprising a significant fragment of such proteins. It also embraces a mouse or human derived polypeptide which exhibits similar biological function or interacts with CCF18 chemokine specific binding components. These binding components, e.g., antibodies, typically bind to a CCF18 chemokine with high affinity, e.g., at least about 100 nM, usually better than about 30 nM, preferably better than about 10 nM, and more preferably at better than about 3 nM. Homologous proteins would be found in mammalian species other than mouse, e.g., rats. Non-mammalian species should also possess structurally or functionally related genes and proteins.

The term "polypeptide" as used herein includes a significant fragment or segment, and encompasses a stretch of amino acid residues of at least about 8 amino acids, generally at least 10 amino acids, more generally at least 12 amino acids, often at least 14 amino acids, more often at least 16 amino acids, typically at least 18 amino acids, more typically at least 20 amino acids, usually at least 22 amino acids, more usually at least 24 amino acids, preferably at least 26 amino acids, more preferably at least 28 amino acids, and, in particularly preferred embodiments, at least about 30 or more amino acids, e.g., about 35, 40, 45, 50, 60, 75, 80, 100, 120, etc.

The term "binding composition" refers to molecules that bind with specificity to CCF18 chemokine, e.g., in a ligand-receptor type fashion or an antibody-antigen interaction. These compositions may be compounds, e.g., proteins, which specifically associate with CCF18 chemokine, including natural physiologically relevant protein-protein interactions, either covalent or non-covalent. The binding composition may be a polymer, or another chemical reagent. No implication as to whether the CCF18 chemokine presents a

11

concave or convex shape in its ligand-receptor interaction is represented, other than the interaction exhibit similar specificity, e.g., specific affinity. A functional analog may be a ligand with structural modifications, or may be a 5 wholly unrelated molecule, e.g., which has a molecular shape which interacts with the appropriate ligand binding determinants. The ligands may serve as agonists or antagonists of the receptor, see, e.g., Goodman, et al. (eds.) (1990) Goodman & Gilman's: The Pharmacological Bases
10 of Therapeutics (8th ed.), Pergamon Press.

Substantially pure means that the protein is free from other contaminating proteins, nucleic acids, and other biologicals typically derived from the original source organism. Purity may be assayed by standard methods, and 15 will ordinarily be at least about 40% pure, more ordinarily at least about 50% pure, generally at least about 60% pure, more generally at least about 70% pure, often at least about 75% pure, more often at least about 80% pure, typically at least about 85% pure, more typically at least about 90% pure, 20 preferably at least about 95% pure, more preferably at least about 98% pure, and in most preferred embodiments, at least 99% pure.

Solubility of a polypeptide or fragment depends upon the environment and the polypeptide. Many parameters affect 25 polypeptide solubility, including temperature, electrolyte environment, size and molecular characteristics of the polypeptide, and nature of the solvent. Typically, the temperature at which the polypeptide is used ranges from about 4° C to about 65° C. Usually the temperature at use is 30 greater than about 18° C and more usually greater than about 22° C. For diagnostic purposes, the temperature will usually be about room temperature or warmer, but less than the denaturation temperature of components in the assay. For therapeutic purposes, the temperature will usually be body 35 temperature, typically about 37° C for humans, though under certain situations the temperature may be raised or lowered in situ or in vitro.

12

The electrolytes will usually approximate in situ physiological conditions, but may be modified to higher or lower ionic strength where advantageous. The actual ions may be modified, e.g., to conform to standard buffers used in 5 physiological or analytical contexts.

The size and structure of the polypeptide should generally be in a substantially stable state, and usually not in a denatured state. The polypeptide may be associated with other polypeptides in a quaternary structure, e.g., to confer 10 solubility, or associated with lipids or detergents in a manner which approximates natural lipid bilayer interactions.

The solvent will usually be a biologically compatible buffer, of a type used for preservation of biological activities, and will usually approximate a physiological 15 solvent. Usually the solvent will have a neutral pH, typically between about 5 and 10, and preferably about 7.5. On some occasions, a detergent will be added, typically a mild non-denaturing one, e.g., CHS or CHAPS, or a low enough concentration as to avoid significant disruption of 20 structural or physiological properties of the ligand.

Solubility is reflected by sedimentation measured in Svedberg units, which are a measure of the sedimentation velocity of a molecule under particular conditions. The determination of the sedimentation velocity was classically 25 performed in an analytical ultracentrifuge, but is typically now performed in a standard ultracentrifuge. See, Freifelder (1982) Physical Biochemistry (2d ed.), W.H. Freeman; and Cantor and Schimmel (1980) Biophysical Chemistry, parts 1-3, W.H. Freeman & Co., San Francisco. As a crude determination, 30 a sample containing a putatively soluble polypeptide is spun in a standard full sized ultracentrifuge at about 50K rpm for about 10 minutes, and soluble molecules will remain in the supernatant. A soluble particle or polypeptide will typically be less than about 30S, more typically less than about 15S, usually less than about 10S, more usually less than about 6S, and, in particular embodiments, preferably less than about 4S, and more preferably less than about 3S.

Physical Variants

This invention also encompasses proteins or peptides having substantial amino acid sequence homology with the amino acid sequence of the CCF18 chemokine. The variants 5 include species or allelic variants.

Amino acid sequence homology, or sequence identity, is determined by optimizing residue matches, if necessary, by introducing gaps as required. This changes when considering conservative substitutions as matches. Conservative 10 substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Homologous amino acid sequences are typically 15 intended to include natural allelic and interspecies variations in each respective protein sequence. Typical homologous proteins or peptides will have from 25-100% homology (if gaps can be introduced), to 50-100% homology (if conservative substitutions are included) with the amino acid 20 sequence of the CCF18 chemokine. Homology measures will be at least about 35%, generally at least 40%, more generally at least 45%, often at least 50%, more often at least 55%, typically at least 60%, more typically at least 65%, usually at least 70%, more usually at least 75%, preferably at least 25 80%, and more preferably at least 80%, and in particularly preferred embodiments, at least 85% or more. See also Needleham, et al. (1970) J. Mol. Biol. 48:443; Sankoff, et al. (1983) Chapter One in Time Warps, String Edits, and Macromolecules: The Theory and Practice of Sequence 30 Comparison Addison-Wesley, Reading, MA; and software packages from IntelliGenetics, Mountain View, CA; and the University of Wisconsin Genetics Computer Group, Madison, WI.

The isolated CCF18 chemokine DNA can be readily modified by nucleotide substitutions, nucleotide deletions, nucleotide 35 insertions, and inversions of nucleotide stretches. These modifications result in novel DNA sequences which encode these antigens, their derivatives, or proteins having similar physiological, immunogenic, or antigenic activity. These

14

modified sequences can be used to produce mutant antigens or to enhance expression. Enhanced expression may involve gene amplification, increased transcription, increased translation, and other mechanisms. Such mutant CCF18 5 chemokine derivatives include predetermined or site-specific mutations of the respective protein or its fragments. "Mutant CCF18 chemokine" encompasses a polypeptide otherwise falling within the homology definition of the mouse CCF18 chemokine as set forth above, but having an amino acid 10 sequence which differs from that of CCF18 chemokine as found in nature, whether by way of deletion, substitution, or insertion. In particular, "site specific mutant CCF18 chemokine" generally includes proteins having significant 15 homology with a ligand having sequences of Table 1 or 3, and as sharing various biological activities, e.g., antigenic or immunogenic, with those sequences, and in preferred embodiments contain most of the disclosed sequences. Similar concepts apply to different CCF18 chemokine proteins, particularly those found in various warm blooded animals, 20 e.g., mammals and birds. As stated before, it is emphasized that descriptions are generally meant to encompass all CCF18 chemokine proteins, not limited to the mouse or human embodiments specifically discussed.

Although site specific mutation sites are predetermined, 25 mutants need not be site specific. CCF18 chemokine mutagenesis can be conducted by making amino acid insertions or deletions. Substitutions, deletions, insertions, or any combinations may be generated to arrive at a final construct. Insertions include amino- or carboxy- terminal fusions. 30 Random mutagenesis can be conducted at a target codon and the expressed mutants can then be screened for the desired activity. Methods for making substitution mutations at predetermined sites in DNA having a known sequence are well known in the art, e.g., by M13 primer mutagenesis or 35 polymerase chain reaction (PCR) techniques. See also Sambrook, et al. (1989) and Ausubel, et al. (1987 and Supplements).

The mutations in the DNA normally should not place coding sequences out of reading frames and preferably will not create complementary regions that could hybridize to produce secondary mRNA structure such as loops or hairpins.

5 The present invention also provides recombinant proteins, e.g., heterologous fusion proteins using segments from these proteins. A heterologous fusion protein is a fusion of proteins or segments which are naturally not normally fused in the same manner. Thus, the fusion product
10 of an immunoglobulin with a CCF18 chemokine polypeptide is a continuous protein molecule having sequences fused in a typical peptide linkage, typically made as a single translation product and exhibiting properties derived from each source peptide. A similar concept applies to
15 heterologous nucleic acid sequences.

In addition, new constructs may be made from combining similar functional domains from other proteins. For example, ligand-binding or other segments may be "swapped" between different new fusion polypeptides or fragments. See, e.g.,
20 Cunningham, et al. (1989) Science 243:1330; and O'Dowd, et al. (1988) J. Biol. Chem. 263:15985. Thus, new chimeric polypeptides exhibiting new combinations of specificities will result from the functional linkage of ligand-binding specificities and other functional domains.

25 The phosphoramidite method described by Beaucage and Carruthers (1981) Tetra. Letts. 22:1859, will produce suitable synthetic DNA fragments. A double stranded fragment will often be obtained either by synthesizing the complementary strand and annealing the strand together under
30 appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence, e.g., PCR techniques.

Functional Variants

35 The blocking of physiological response to CCF18 chemokines may result from the inhibition of binding of the ligand to its receptor, likely through competitive inhibition. Thus, in vitro assays of the present invention

¹⁶

will often use isolated protein, membranes from cells expressing a recombinant membrane associated CCF18 chemokine, soluble fragments comprising receptor binding segments of these ligands, or fragments attached to solid phase substrates. These assays will also allow for the diagnostic determination of the effects of either binding segment mutations and modifications, or ligand mutations and modifications, e.g., ligand analogs.

This invention also contemplates the use of competitive drug screening assays, e.g., where neutralizing antibodies to antigen or receptor fragments compete with a test compound for binding to the protein. In this manner, the antibodies can be used to detect the presence of any polypeptide which shares one or more antigenic binding sites of the ligand and can also be used to occupy binding sites on the protein that might otherwise interact with a receptor.

Additionally, neutralizing antibodies against CCF18 chemokine and soluble fragments of the chemokine which contain a high affinity receptor binding site, can be used to inhibit chemokine activity in tissues, e.g., tissues experiencing abnormal physiology.

"Derivatives" of CCF18 chemokine antigens include amino acid sequence mutants, glycosylation variants, and covalent or aggregate conjugates with other chemical moieties. Covalent derivatives can be prepared by linkage of functionalities to groups which are found in CCF18 chemokine amino acid side chains or at the N- or C- termini, by means which are well known in the art. These derivatives can include, without limitation, aliphatic esters or amides of the carboxyl terminus, or of residues containing carboxyl side chains, O-acyl derivatives of hydroxyl group-containing residues, and N-acyl derivatives of the amino terminal amino acid or amino-group containing residues, e.g., lysine or arginine. Acyl groups are selected from the group of alkyl-moieties including C3 to C18 normal alkyl, thereby forming alkanoyl aroyl species. Covalent attachment to carrier proteins may be important when immunogenic moieties are haptens.

In particular, glycosylation alterations are included, e.g., made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing, or in further processing steps. Particularly preferred means for 5 accomplishing this are by exposing the polypeptide to glycosylating enzymes derived from cells which normally provide such processing, e.g., mammalian glycosylation enzymes. Deglycosylation enzymes are also contemplated. Also embraced are versions of the same primary amino acid 10 sequence which have other minor modifications, including phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, or phosphothreonine.

A major group of derivatives are covalent conjugates of the CCF18 chemokine or fragments thereof with other proteins 15 or polypeptides. These derivatives can be synthesized in recombinant culture such as N- or C-terminal fusions or by the use of agents known in the art for their usefulness in cross-linking proteins through reactive side groups. Preferred chemokine derivatization sites with cross-linking 20 agents are at free amino groups, carbohydrate moieties, and cysteine residues.

Fusion polypeptides between CCF18 chemokines and other homologous or heterologous proteins, e.g., other chemokines, are also provided. Many growth factors and cytokines are 25 homodimeric entities, and a repeat construct may have various advantages, including lessened susceptibility to proteolytic cleavage. Moreover, many receptors require dimerization to transduce a signal, and various dimeric ligands or domain repeats can be desirable. Homologous polypeptides may be 30 fusions between different surface markers, resulting in, e.g., a hybrid protein exhibiting receptor binding specificity. Likewise, heterologous fusions may be constructed which would exhibit a combination of properties or activities of the derivative proteins. Typical examples 35 are fusions of a reporter polypeptide, e.g., luciferase, with a segment or domain of a ligand, e.g., a receptor-binding segment, so that the presence or location of the fused ligand may be easily determined. See, e.g., Dull, et al., U.S.

/8

Patent No. 4,859,609. Other gene fusion partners include bacterial β -galactosidase, trpE, Protein A, β -lactamase, alpha amylase, alcohol dehydrogenase, a FLAG fusion, and yeast alpha mating factor. See, e.g., Godowski, et al. 5 (1988) Science 241:812.

The phosphoramidite method described by Beaucage and Carruthers (1981) Tetra. Letts. 22:1859, will produce suitable synthetic DNA fragments. A double stranded fragment will often be obtained either by synthesizing the 10 complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Such polypeptides may also have amino acid residues which have been chemically modified by phosphorylation, 15 sulfonation, biotinylation, or the addition or removal of other moieties, particularly those which have molecular shapes similar to phosphate groups. In some embodiments, the modifications will be useful labeling reagents, or serve as purification targets, e.g., affinity tags as FLAG.

20 Fusion proteins will typically be made by either recombinant nucleic acid methods or by synthetic polypeptide methods. Techniques for nucleic acid manipulation and expression are described generally, for example, in Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual (2d 25 ed.), Vols. 1-3, Cold Spring Harbor Laboratory. Techniques for synthesis of polypeptides are described, for example, in Merrifield (1963) J. Amer. Chem. Soc. 85:2149; Merrifield (1986) Science 232:341; and Atherton, et al. (1989) Solid 30 Phase Peptide Synthesis: A Practical Approach, IRL Press, Oxford.

This invention also contemplates the use of derivatives of CCF18 chemokines other than variations in amino acid sequence or glycosylation. Such derivatives may involve covalent or aggregative association with chemical moieties. 35 These derivatives generally fall into the three classes: (1) salts, (2) side chain and terminal residue covalent modifications, and (3) adsorption complexes, for example with cell membranes. Such covalent or aggregative derivatives are

19

useful as immunogens, as reagents in immunoassays, or in purification methods such as for affinity purification of ligands or other binding ligands. For example, a CCF18 chemokine antigen can be immobilized by covalent bonding to a 5 solid support such as cyanogen bromide-activated Sepharose, by methods which are well known in the art, or adsorbed onto polyolefin surfaces, with or without glutaraldehyde cross-linking, for use in the assay or purification of anti-CCF18 chemokine antibodies or its receptor. The CCF18 chemokines 10 can also be labeled with a detectable group, for example radioiodinated by the chloramine T procedure, covalently bound to rare earth chelates, or conjugated to another fluorescent moiety for use in diagnostic assays. Purification of CCF18 chemokine may be effected by 15 immobilized antibodies or receptor.

A solubilized CCF18 chemokine or fragment of this invention can be used as an immunogen for the production of antisera or antibodies specific for the ligand or fragments thereof. The purified chemokines can be used to screen 20 monoclonal antibodies or chemokine-binding fragments prepared by immunization with various forms of impure preparations containing the protein. In particular, the term "antibodies" also encompasses antigen binding fragments of natural antibodies. Purified CCF18 chemokine can also be used as a 25 reagent to detect antibodies generated in response to the presence of elevated levels of the protein or cell fragments containing the protein, both of which may be diagnostic of an abnormal or specific physiological or disease condition. Additionally, chemokine protein fragments may also serve as 30 immunogens to produce antibodies of the present invention, as described immediately below. For example, this invention contemplates antibodies raised against amino acid sequences shown in Tables 1 or 3, or proteins containing them. In particular, this invention contemplates antibodies having 35 binding affinity to or being raised against specific fragments, e.g., those which are predicted to lie on the outside surfaces of protein tertiary structure.

20

The present invention contemplates the isolation of additional closely related species variants. Southern and Northern blot analysis should establish that similar genetic entities exist in other mammals. It is likely that CCF18 chemokines are widespread in species variants, e.g., rodents, lagomorphs, carnivores, artiodactyla, perissodactyla, and primates.

5 The invention also provides means to isolate a group of related chemokines displaying both distinctness and 10 similarities in structure, expression, and function. Elucidation of many of the physiological effects of the proteins will be greatly accelerated by the isolation and 15 characterization of distinct species variants of the ligands. In particular, the present invention provides useful probes for identifying additional homologous genetic entities in different species.

The isolated genes will allow transformation of cells 20 lacking expression of a corresponding CCF18 chemokine, e.g., either species types or cells which lack corresponding ligands and exhibit negative background activity. Expression 25 of transformed genes will allow isolation of antigenically pure cell lines, with defined or single specie variants. This approach will allow for more sensitive detection and discrimination of the physiological effects of CCF18 receptor proteins. Subcellular fragments, e.g., cytoplasts or membrane fragments, can be isolated and used.

Dissection of critical structural elements which effect 30 the various differentiation functions provided by ligands is possible using standard techniques of modern molecular biology, particularly in comparing members of the related class. See, e.g., the homolog-scanning mutagenesis technique described in Cunningham, et al. (1989) Science 243:1339; and approaches used in O'Dowd, et al. (1988) J. Biol. Chem. 263:15985; and Lechleiter, et al. (1990) EMBO J. 9:4381.

35 In particular, receptor binding segments can be substituted between species variants to determine what structural features are important in both receptor binding affinity and specificity, as well as signal transduction. An

21

array of different chemokine variants will be used to screen for ligands exhibiting combined properties of interaction with different receptor species variants.

Intracellular functions would probably involve segments 5 of the receptor which are normally accessible to the cytosol. However, ligand internalization may occur under certain circumstances, and interaction between intracellular components and "extracellular" segments may occur. The specific segments of interaction of CCF18 chemokine with 10 other intracellular components may be identified by mutagenesis or direct biochemical means, e.g., cross-linking or affinity methods. Structural analysis by crystallographic or other physical methods will also be applicable. Further investigation of the mechanism of signal transduction will 15 include study of associated components which may be isolatable by affinity methods or by genetic means, e.g., complementation analysis of mutants.

Further study of the expression and control of CCF18 chemokine will be pursued. The controlling elements 20 associated with the proteins may exhibit differential developmental, tissue specific, or other expression patterns. Upstream or downstream genetic regions, e.g., control elements, are of interest. Differential splicing of message may lead to membrane bound forms, soluble forms, and modified 25 versions of ligand.

Structural studies of the proteins will lead to design of new ligands, particularly analogs exhibiting agonist or antagonist properties on the receptor. This can be combined with previously described screening methods to isolate 30 ligands exhibiting desired spectra of activities.

Expression in other cell types will often result in glycosylation differences in a particular chemokine. Various species variants may exhibit distinct functions based upon structural differences other than amino acid sequence. 35 Differential modifications may be responsible for differential function, and elucidation of the effects are now made possible.

22

Thus, the present invention provides important reagents related to a physiological chemokine-binding protein interaction. Although the foregoing description has focused primarily upon the mouse and human CCF18 chemokine, those of skill in the art will immediately recognize that the invention encompasses other species counterparts, e.g., rat and other mammalian species or allelic variants, as well as variants thereof.

10 Antibodies

Antibodies can be raised to CCF18 chemokines, including species or allelic variants, and fragments thereof, both in their naturally occurring forms and in their recombinant forms. Additionally, antibodies can be raised to CCF18 chemokines in either their active forms or in their inactive forms. Anti-idiotypic antibodies are also contemplated.

Antibodies, including binding fragments and single chain versions, against predetermined fragments of the ligands can be raised by immunization of animals with conjugates of the fragments with immunogenic proteins. Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies can be screened for binding to normal or defective CCF18 chemokines, or screened for agonistic or antagonistic activity, e.g., mediated through a receptor for CCF18. These monoclonal antibodies will usually bind with at least a K_D of about 1 mM, more usually at least about 300 μ M, typically at least about 10 μ M, more typically at least about 30 μ M, preferably at least about 10 μ M, and more preferably at least about 3 μ M or better.

30 The antibodies, including antigen binding fragments, of this invention can have significant diagnostic or therapeutic value. They can be potent antagonists that bind to a receptor and inhibit ligand binding or inhibit the ability of a ligand to elicit a biological response. They also can be 35 useful as non-neutralizing antibodies and can be coupled to toxins or radionuclides so that when the antibody binds to ligand, a cell expressing it, e.g., on its surface, is killed. Further, these antibodies can be conjugated to drugs

23

or other therapeutic agents, either directly or indirectly by means of a linker, and may effect drug targeting.

The antibodies of this invention can also be useful in diagnostic applications. As capture or non-neutralizing 5 antibodies, they can be screened for ability to bind to the chemokines without inhibiting receptor binding. As neutralizing antibodies, they can be useful in competitive binding assays. They will also be useful in detecting or quantifying CCF18 chemokine or, indirectly, receptors.

10 Ligand fragments may be joined to other materials, particularly polypeptides, as fused or covalently joined polypeptides to be used as immunogens. A ligand and its fragments may be fused or covalently linked to a variety of immunogens, such as keyhole limpet hemocyanin, bovine serum 15 albumin, tetanus toxoid, etc. See Microbiology, Hoeber Medical Division, Harper and Row, 1969; Landsteiner (1962) Specificity of Serological Reactions, Dover Publications, New York, and Williams, et al. (1967) Methods in Immunology and Immunochemistry, Vol. 1, Academic Press, New York, for 20 descriptions of methods of preparing polyclonal antisera. A typical method involves hyperimmunization of an animal with an antigen. The blood of the animal is then collected shortly after the repeated immunizations and the gamma globulin is isolated.

25 In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice, rodents, primates, humans, etc. Description of techniques for preparing such monoclonal antibodies may be found in, e.g., Stites, et al. (eds.) Basic and Clinical Immunology 30 (4th ed.), Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH Press; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed.) Academic Press, New York; and particularly in Kohler and Milstein (1975) in 35 Nature 256:495, which discusses one method of generating monoclonal antibodies. Summarized briefly, this method involves injecting an animal with an immunogen. The animal is then sacrificed and cells taken from its spleen, which are

then fused with myeloma cells. The result is a hybrid cell or "hybridoma" that is capable of reproducing in vitro. The population of hybridomas is then screened to isolate individual clones, each of which secrete a single antibody 5 species to the immunogen. In this manner, the individual antibody species obtained are the products of immortalized and cloned single B cells from the immune animal generated in response to a specific site recognized on the immunogenic substance.

10 Other suitable techniques involve in vitro exposure of lymphocytes to the antigenic polypeptides or alternatively to selection of libraries of antibodies in phage or similar vectors. See, Huse, et al. (1989) "Generation of a Large Combinatorial Library of the Immunoglobulin Repertoire in 15 Phage Lambda," Science 246:1275; and Ward, et al. (1989) Nature 341:544. The polypeptides and antibodies of the present invention may be used with or without modification, including chimeric or humanized antibodies. Frequently, the polypeptides and antibodies will be labeled by joining, 20 either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, 25 cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents, teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins 30 may be produced, see Cabilly, U.S. Patent No. 4,816,567; and Queen, et al. (1989) Proc. Nat'l. Acad. Sci. 86:10029.

The antibodies of this invention can also be used for affinity chromatography in isolating the protein. Columns can be prepared where the antibodies are linked to a solid 35 support, e.g., particles, such as agarose, Sephadex, or the like, where a cell lysate may be passed through the column, the column washed, followed by increasing concentrations of a

25

mild denaturant, whereby the purified CCF18 chemokine protein will be released.

5 The antibodies may also be used to screen expression libraries for particular expression products. Usually the antibodies used in such a procedure will be labeled with a moiety allowing easy detection of presence of antigen by antibody binding.

10 Antibodies raised against CCF18 chemokine will also be useful to raise anti-idiotypic antibodies. These will be useful in detecting or diagnosing various immunological conditions related to expression of the respective antigens.

Nucleic Acids

15 The described peptide sequences and the related reagents are useful in isolating a DNA clone encoding CCF18 chemokine, e.g., from a natural source. Typically, it will be useful in isolating a gene from mouse, and similar procedures will be applied to isolate genes from other species, e.g., warm blooded animals, such as birds and mammals. Cross 20 hybridization will allow isolation of ligand from other species. A number of different approaches should be available to successfully isolate a suitable nucleic acid clone.

25 The purified protein or defined peptides are useful for generating antibodies by standard methods, as described above. Synthetic peptides or purified protein can be presented to an immune system to generate monoclonal or polyclonal antibodies. See, e.g., Coligan (1991) Current Protocols in Immunology Wiley/Greene; and Harlow and Lane 30 (1989) Antibodies: A Laboratory Manual Cold Spring Harbor Press. Alternatively, a CCF18 receptor can be used as a specific binding reagent, and advantage can be taken of its specificity of binding, much like an antibody would be used. However, chemokine receptors are typically 7 transmembrane 35 proteins, which could be sensitive to appropriate interaction with lipid or membrane.

For example, the specific binding composition could be used for screening of an expression library made from a cell

26

line which expresses a CCF18 chemokine. The screening can be standard staining of surface expressed ligand, or by panning. Screening of intracellular expression can also be performed by various staining or immunofluorescence procedures. The 5 binding compositions could be used to affinity purify or sort out cells expressing the ligand.

The peptide segments can also be used to predict appropriate oligonucleotides to screen a library, e.g., to isolate species variants. The genetic code can be used to 10 select appropriate oligonucleotides useful as probes for screening. See, e.g., Table 1 or 3. In combination with polymerase chain reaction (PCR) techniques, synthetic oligonucleotides will be useful in selecting correct clones from a library. Complementary sequences will also be used as 15 probes or primers. Based upon identification of the likely amino terminus, the third peptide should be particularly useful, e.g., coupled with anchored vector or poly-A complementary PCR techniques or with complementary DNA of other peptides.

20 This invention contemplates use of isolated DNA or fragments to encode a biologically active corresponding CCF18 chemokine polypeptide. In addition, this invention covers isolated or recombinant DNA which encodes a biologically active protein or polypeptide which is capable of hybridizing 25 under appropriate conditions with the DNA sequences described herein. Said biologically active protein or polypeptide can be an intact ligand, or fragment, and have an amino acid sequence as disclosed in Table 1 or 3. Further, this invention covers the use of isolated or recombinant DNA, or 30 fragments thereof, which encode proteins which are homologous to a CCF18 chemokine or which was isolated using cDNA encoding a CCF18 chemokine as a probe. The isolated DNA can have the respective regulatory sequences in the 5' and 3' flanks, e.g., promoters, enhancers, poly-A addition signals, 35 and others.

An "isolated" nucleic acid is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially separated from other components which naturally accompany a

27

native sequence, e.g., ribosomes, polymerases, and flanking genomic sequences from the originating species. The term embraces a nucleic acid sequence which has been removed from its naturally occurring environment, and includes recombinant 5 or cloned DNA isolates and chemically synthesized analogs or analogs biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule.

An isolated nucleic acid will generally be a homogeneous 10 composition of molecules, but will, in some embodiments, contain minor heterogeneity. This heterogeneity is typically found at the polymer ends or portions not critical to a desired biological function or activity.

A "recombinant" nucleic acid is defined either by its 15 method of production or its structure. In reference to its method of production, e.g., a product made by a process, the process is use of recombinant nucleic acid techniques, e.g., involving human intervention in the nucleotide sequence, typically selection or production. Alternatively, it can be 20 a nucleic acid made by generating a sequence comprising fusion of two fragments which are not naturally contiguous to each other, but is meant to exclude products of nature, e.g., naturally occurring mutants. Thus, for example, products made by transforming cells with any unnaturally occurring 25 vector is encompassed, as are nucleic acids comprising sequence derived using any synthetic oligonucleotide process. Such is often done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition 30 site. Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a single genetic entity comprising a desired combination of functions not found in the commonly available natural forms. Restriction enzyme recognition sites are often the target of 35 such artificial manipulations, but other site specific targets, e.g., promoters, DNA replication sites, regulation sequences, control sequences, or other useful features may be incorporated by design. A similar concept is intended for a

recombinant, e.g., fusion, polypeptide. Specifically included are synthetic nucleic acids which, by genetic code redundancy, encode polypeptides similar to fragments of these antigens, and fusions of sequences from various different 5 species variants.

A significant "fragment" in a nucleic acid context is a contiguous segment of at least about 17 nucleotides, generally at least about 20 nucleotides, more generally at least about 23 nucleotides, ordinarily at least about 26 10 nucleotides, more ordinarily at least about 29 nucleotides, often at least about 32 nucleotides, more often at least about 35 nucleotides, typically at least about 38 nucleotides, more typically at least about 41 nucleotides, usually at least about 44 nucleotides, more usually at least 15 about 47 nucleotides, preferably at least about 50 nucleotides, more preferably at least about 53 nucleotides, and in particularly preferred embodiments will be at least about 56 or more nucleotides, e.g., 60, 65, 75, 85, 100, 120, 150, 200, 250, 300, 400, etc.

20 A DNA which codes for a CCF18 chemokine protein or peptide will be particularly useful to identify genes, mRNA, and cDNA species which code for related or homologous ligands, as well as DNAs which code for homologous proteins from different species. There are likely homologs in other 25 species, including primates. Various CCF18 chemokine proteins should be homologous and are encompassed herein. However, even proteins that have a more distant evolutionary relationship to the ligand can readily be isolated under appropriate conditions using these sequences if they are 30 sufficiently homologous. Primate CCF18 chemokines are of particular interest.

This invention further covers recombinant DNA molecules and fragments having a DNA sequence identical to or highly homologous to the isolated DNAs set forth herein. In 35 particular, the sequences will often be operably linked to DNA segments which control transcription, translation, and DNA replication. Alternatively, recombinant clones derived from the genomic sequences, e.g., containing introns, will be

useful for transgenic studies, including, e.g., transgenic cells and organisms, and for gene therapy. See, e.g., Goodnow (1992) "Transgenic Animals" in Roitt (ed.) Encyclopedia of Immunology Academic Press, San Diego, pp. 5 1502-1504; Travis (1992) Science 256:1392; Kuhn, et al. (1991) Science 254:707; Capecchi (1989) Science 244:1288; Robertson (1987) (ed.) Teratocarcinomas and Embryonic Stem Cells: A Practical Approach IRL Press, Oxford; and Rosenberg (1992) J. Clinical Oncology 10:180.

10 Homologous nucleic acid sequences, when compared, exhibit significant similarity. The standards for homology in nucleic acids are either measures for homology generally used in the art by sequence comparison or based upon hybridization conditions. The hybridization conditions are 15 described in greater detail below.

Substantial homology in the nucleic acid sequence comparison context means either that the segments, or their complementary strands, when compared, are identical when optimally aligned, with appropriate nucleotide insertions or 20 deletions, in at least about 50% of the nucleotides, generally at least about 56%, more generally at least about 59%, ordinarily at least about 62%, more ordinarily at least about 65%, often at least about 68%, more often at least about 71%, typically at least about 74%, more typically at 25 least about 77%, usually at least about 80%, more usually at least about 85%, preferably at least about 90%, more preferably at least about 95 to 98% or more, and in particular embodiments, as high as about 99% or more of the nucleotides. Alternatively, substantial homology exists when 30 the segments will hybridize under selective hybridization conditions, to a strand, or its complement, typically using a sequence derived from Table 1 or 3. Typically, selective hybridization will occur when there is at least about 55% homology over a stretch of at least about 30 nucleotides, 35 preferably at least about 65% over a stretch of at least about 25 nucleotides, more preferably at least about 75%, and most preferably at least about 90% over about 20 nucleotides. See, Kanehisa (1984) Nuc. Acids Res. 12:203. The length of

30

homology comparison, as described, may be over longer stretches, and in certain embodiments will be over a stretch of at least about 17 nucleotides, usually at least about 20 nucleotides, more usually at least about 24 nucleotides, 5 typically at least about 28 nucleotides, more typically at least about 40 nucleotides, preferably at least about 50 nucleotides, and more preferably at least about 75 to 100 or more nucleotides.

Stringent conditions, in referring to homology in the 10 hybridization context, will be stringent combined conditions of salt, temperature, organic solvents, and other parameters, typically those controlled in hybridization reactions. Stringent temperature conditions will usually include temperatures in excess of about 30° C, more usually in excess 15 of about 37° C, typically in excess of about 45° C, more typically in excess of about 55° C, preferably in excess of about 65° C, and more preferably in excess of about 70° C. Stringent salt conditions will ordinarily be less than about 1000 mM, usually less than about 500 mM, more usually less 20 than about 400 mM, typically less than about 300 mM, preferably less than about 200 mM, and more preferably less than about 150 mM. However, the combination of parameters is much more important than the measure of any single parameter. See, e.g., Wetmur and Davidson (1968) J. Mol. Biol. 31:349.

25 CCF18 chemokine from other mammalian species can be cloned and isolated by cross-species hybridization of closely related species. Alternatively, sequences from a data base may be recognized as having similarity. Homology may be relatively low between distantly related species, and thus 30 hybridization of relatively closely related species is advisable. Alternatively, preparation of an antibody preparation which exhibits less species specificity may be useful in expression cloning approaches.

35 Making CCF18 chemokine: Mimetics

DNA which encodes the CCF18 chemokine or fragments thereof can be obtained by chemical synthesis, screening cDNA

31

libraries, or by screening genomic libraries prepared from a wide variety of cell lines or tissue samples.

This DNA can be expressed in a wide variety of host cells for the synthesis of a full-length ligand or fragments which can in turn, for example, be used to generate polyclonal or monoclonal antibodies; for binding studies; for construction and expression of modified molecules; and for structure/function studies. Each antigen or its fragments can be expressed in host cells that are transformed or transfected with appropriate expression vectors. These molecules can be substantially purified to be free of protein or cellular contaminants, other than those derived from the recombinant host, and therefore are particularly useful in pharmaceutical compositions when combined with a pharmaceutically acceptable carrier and/or diluent. The antigen, or portions thereof, may be expressed as fusions with other proteins.

Expression vectors are typically self-replicating DNA or RNA constructs containing the desired antigen gene or its fragments, usually operably linked to suitable genetic control elements that are recognized in a suitable host cell. These control elements are capable of effecting expression within a suitable host. The specific type of control elements necessary to effect expression will depend upon the eventual host cell used. Generally, the genetic control elements can include a prokaryotic promoter system or a eukaryotic promoter expression control system, and typically include a transcriptional promoter, an optional operator to control the onset of transcription, transcription enhancers to elevate the level of mRNA expression, a sequence that encodes a suitable ribosome binding site, and sequences that terminate transcription and translation. Expression vectors also usually contain an origin of replication that allows the vector to replicate independently of the host cell.

The vectors of this invention contain DNA which encodes a CCF18 chemokine, or a fragment thereof, typically encoding a biologically active polypeptide. The DNA can be under the control of a viral promoter and can encode a selection

marker. This invention further contemplates use of such expression vectors which are capable of expressing eukaryotic cDNA coding for a CCF18 chemokine in a prokaryotic or eukaryotic host, where the vector is compatible with the host and where the eukaryotic cDNA coding for the ligand is inserted into the vector such that growth of the host containing the vector expresses the cDNA in question. Usually, expression vectors are designed for stable replication in their host cells or for amplification to greatly increase the total number of copies of the desirable gene per cell. It is not always necessary to require that an expression vector replicate in a host cell, e.g., it is possible to effect transient expression of the ligand or its fragments in various hosts using vectors that do not contain a replication origin that is recognized by the host cell. It is also possible to use vectors that cause integration of a CCF18 chemokine gene or its fragments into the host DNA by recombination, or to integrate a promoter which controls expression of an endogenous gene.

Vectors, as used herein, comprise plasmids, viruses, bacteriophage, integratable DNA fragments, and other vehicles which enable the integration of DNA fragments into the genome of the host. Expression vectors are specialized vectors which contain genetic control elements that effect expression of operably linked genes. Plasmids are the most commonly used form of vector but all other forms of vectors which serve an equivalent function and which are, or become, known in the art are suitable for use herein. See, e.g., Pouwels, et al. (1985 and Supplements) Cloning Vectors: A Laboratory Manual, Elsevier, N.Y., and Rodriguez, et al. (1988) (eds.) Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworth, Boston, MA.

Transformed cells include cells, preferably mammalian, that have been transformed or transfected with CCF18 chemokine gene containing vectors constructed using recombinant DNA techniques. Transformed host cells usually express the ligand or its fragments, but for purposes of cloning, amplifying, and manipulating its DNA, do not need to

express the protein. This invention further contemplates culturing transformed cells in a nutrient medium, thus permitting the protein to accumulate in the culture. The protein can be recovered, either from the culture or from the 5 culture medium.

For purposes of this invention, DNA sequences are operably linked when they are functionally related to each other. For example, DNA for a presequence or secretory leader is operably linked to a polypeptide if it is expressed 10 as a preprotein or participates in directing the polypeptide to the cell membrane or in secretion of the polypeptide. A promoter is operably linked to a coding sequence if it controls the transcription of the polypeptide; a ribosome binding site is operably linked to a coding sequence if it is 15 positioned to permit translation. Usually, operably linked means contiguous and in reading frame, however, certain genetic elements such as repressor genes are not contiguously linked but still bind to operator sequences that in turn control expression.

20 Suitable host cells include prokaryotes, lower eukaryotes, and higher eukaryotes. Prokaryotes include both gram negative and gram positive organisms, e.g., *E. coli* and *B. subtilis*. Lower eukaryotes include yeasts, e.g., *S. cerevisiae* and *Pichia*, and species of the genus 25 *Dictyostelium*. Higher eukaryotes include established tissue culture cell lines from animal cells, both of non-mammalian origin, e.g., insect cells, and birds, and of mammalian origin, e.g., human, primates, and rodents.

30 Prokaryotic host-vector systems include a wide variety of vectors for many different species. As used herein, *E. coli* and its vectors will be used generically to include equivalent vectors used in other prokaryotes. A representative vector for amplifying DNA is pBR322 or many of its derivatives. Vectors that can be used to express the 35 CCF18 chemokines or its fragments include, but are not limited to, such vectors as those containing the lac promoter (pUC-series); trp promoter (pBR322-trp); Ipp promoter (the pIN-series); lambda-pP or pR promoters (pOTS); or hybrid

promoters such as ptac (pDR540). See Brosius, et al. (1988) "Expression Vectors Employing Lambda-, trp-, lac-, and Ipp-derived Promoters", in Rodriguez and Denhardt (eds.) Vectors: A Survey of Molecular Cloning Vectors and Their Uses, 5 Buttersworth, Boston, Chapter 10, pp. 205-236.

Lower eukaryotes, e.g., yeasts and Dictyostelium, may be transformed with CCF18 chemokine sequence containing vectors. For purposes of this invention, the most common lower eukaryotic host is the baker's yeast, *Saccharomyces cerevisiae*. It will be used to generically represent lower eukaryotes although a number of other strains and species are also available. Yeast vectors typically consist of a replication origin (unless of the integrating type), a selection gene, a promoter, DNA encoding the desired protein or its fragments, and sequences for translation termination, polyadenylation, and transcription termination. Suitable expression vectors for yeast include such constitutive promoters as 3-phosphoglycerate kinase and various other glycolytic enzyme gene promoters or such inducible promoters as the alcohol dehydrogenase 2 promoter or metallothionein promoter. Suitable vectors include derivatives of the following types: self-replicating low copy number (such as the YRp-series), self-replicating high copy number (such as the YEp-series); integrating types (such as the YIp-series), 25 or mini-chromosomes (such as the YCp-series).

Higher eukaryotic tissue culture cells are the preferred host cells for expression of the functionally active CCF18 chemokine protein. In principle, any higher eukaryotic tissue culture cell line is workable, e.g., insect 30 baculovirus expression systems, whether from an invertebrate or vertebrate source. However, mammalian cells are preferred, in that the processing, both cotranslationally and posttranslationally. Transformation or transfection and propagation of such cells has become a routine procedure. 35 Examples of useful cell lines include HeLa cells, Chinese hamster ovary (CHO) cell lines, baby rat kidney (BRK) cell lines, insect cell lines, bird cell lines, and monkey (COS) cell lines. Expression vectors for such cell lines usually

include an origin of replication, a promoter, a translation initiation site, RNA splice sites (if genomic DNA is used), a polyadenylation site, and a transcription termination site. These vectors also usually contain a selection gene or 5 amplification gene. Suitable expression vectors may be plasmids, viruses, or retroviruses carrying promoters derived, e.g., from such sources as from adenovirus, SV40, parvoviruses, vaccinia virus, or cytomegalovirus. Representative examples of suitable expression vectors 10 include pCDNA1; pCD, see Okayama, et al. (1985) Mol. Cell Biol. 5:1136; pMC1neo Poly-A, see Thomas, et al. (1987) Cell 51:503; and a baculovirus vector such as pAC 373 or pAC 610.

It will often be desired to express a CCF18 chemokine polypeptide in a system which provides a specific or defined 15 glycosylation pattern. In this case, the usual pattern will be that provided naturally by the expression system. However, the pattern will be modifiable by exposing the polypeptide, e.g., an unglycosylated form, to appropriate 20 glycosylating proteins introduced into a heterologous expression system. For example, the CCF18 chemokine gene may be co-transformed with one or more genes encoding mammalian or other glycosylating enzymes. Using this approach, certain mammalian glycosylation patterns will be achievable or approximated in prokaryote or other cells.

25 A CCF18 chemokine, or a fragment thereof, may be engineered to be phosphatidyl inositol (PI) linked to a cell membrane, but can be removed from membranes by treatment with a phosphatidyl inositol cleaving enzyme, e.g., phosphatidyl inositol phospholipase-C. This releases the antigen in a 30 biologically active form, and allows purification by standard procedures of protein chemistry. See, e.g., Low (1989) Biochim. Biophys. Acta 988:427; Tse, et al. (1985) Science 230:1003; and Brunner, et al. (1991) J. Cell Biol. 114:1275.

Now that the CCF18 chemokine has been characterized, 35 fragments or derivatives thereof can be prepared by conventional processes for synthesizing peptides. These include processes such as are described in Stewart and Young (1984) Solid Phase Peptide Synthesis, Pierce Chemical Co.,

Rockford, IL; Bodanszky and Bodanszky (1984) The Practice of Peptide Synthesis, Springer-Verlag, New York; and Bodanszky (1984) The Principles of Peptide Synthesis, Springer-Verlag, New York. For example, an azide process, an acid chloride process, an acid anhydride process, a mixed anhydride process, an active ester process (for example, p-nitrophenyl ester, N-hydroxysuccinimide ester, or cyanomethyl ester), a carbodiimidazole process, an oxidative-reductive process, or a dicyclohexylcarbodiimide (DCCD)/additive process can be used. Solid phase and solution phase syntheses are both applicable to the foregoing processes.

The CCF18 chemokine, fragments, or derivatives are suitably prepared in accordance with the above processes as typically employed in peptide synthesis, generally either by a so-called stepwise process which comprises condensing an amino acid to the terminal amino acid, one by one in sequence, or by coupling peptide fragments to the terminal amino acid. Amino groups that are not being used in the coupling reaction are typically protected to prevent coupling at an incorrect location.

If a solid phase synthesis is adopted, the C-terminal amino acid is bound to an insoluble carrier or support through its carboxyl group. The insoluble carrier is not particularly limited as long as it has a binding capability to a reactive carboxyl group. Examples of such insoluble carriers include halomethyl resins, such as chloromethyl resin or bromomethyl resin, hydroxymethyl resins, phenol resins, tert-alkyloxycarbonyl-hydrazidated resins, and the like.

An amino group-protected amino acid is bound in sequence through condensation of its activated carboxyl group and the reactive amino group of the previously formed peptide or chain, to synthesize the peptide step by step. After synthesizing the complete sequence, the peptide is split off from the insoluble carrier to produce the peptide. This solid-phase approach is generally described by Merrifield, et al. (1963) in J. Am. Chem. Soc. 85:2149.

The prepared ligand and fragments thereof can be isolated and purified from the reaction mixture by means of peptide separation, e.g., by extraction, precipitation, electrophoresis and various forms of chromatography, and the like. The CCF18 chemokines of this invention can be obtained in varying degrees of purity depending upon its desired use. Purification can be accomplished by use of the protein purification techniques disclosed herein or by the use of the antibodies herein described in immunoabsorbant affinity chromatography. This immunoabsorbant affinity chromatography is carried out by first linking the antibodies to a solid support and then contacting the linked antibodies with solubilized lysates of appropriate source cells, lysates of other cells expressing the ligand, or lysates or supernatants of cells producing the CCF18 chemokine as a result of DNA techniques, see below.

Uses

The present invention provides reagents which will find use in diagnostic applications as described elsewhere herein, e.g., in the general description for developmental abnormalities, or below in the description of kits for diagnosis.

This invention also provides reagents with significant therapeutic value. The CCF18 chemokine (naturally occurring or recombinant), fragments thereof and antibodies thereto, along with compounds identified as having binding affinity to CCF18 chemokine, should be useful in the treatment of conditions associated with abnormal physiology or development, including inflammatory conditions. In particular, modulation of trafficking of leukocytes is likely, but a wider tissue distribution might suggest broader biological activity, including, e.g., antiviral effects. Abnormal proliferation, regeneration, degeneration, and atrophy may be modulated by appropriate therapeutic treatment using the compositions provided herein. For example, a disease or disorder associated with abnormal expression or

abnormal signaling by a CCF18 chemokine should be a likely target for an agonist or antagonist of the ligand.

Various abnormal physiological or developmental conditions are known in cell types shown to possess CCF18

5 mRNA by Northern blot analysis. See Berkow (ed.) The Merck Manual of Diagnosis and Therapy, Merck & Co., Rahway, N.J.; and Thorn, et al. Harrison's Principles of Internal Medicine, McGraw-Hill, N.Y. Developmental or functional abnormalities, e.g., of the immune system, cause significant medical
10 abnormalities and conditions which may be susceptible to prevention or treatment using compositions provided herein.

Recombinant CCF18 chemokine antibodies can be purified and then administered to a patient. These reagents can be combined for therapeutic use with additional active or inert
15 ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents, e.g., immunogenic adjuvants, along with physiologically innocuous stabilizers and excipients. These combinations can be sterile filtered and placed into dosage forms as by lyophilization in dosage vials
20 or storage in stabilized aqueous preparations. This invention also contemplates use of antibodies or binding fragments thereof, including forms which are not complement binding.

Drug screening using antibodies or receptor or fragments
25 thereof can be performed to identify compounds having binding affinity to CCF18 chemokine, including isolation of associated components. Subsequent biological assays can then be utilized to determine if the compound has intrinsic stimulating activity and is therefore a blocker or antagonist
30 in that it blocks the activity of the ligand. Likewise, a compound having intrinsic stimulating activity can activate the receptor and is thus an agonist in that it simulates the activity of CCF18 chemokine. This invention further contemplates the therapeutic use of antibodies to CCF18
35 chemokine as antagonists. This approach should be particularly useful with other CCF18 chemokine species variants.

The quantities of reagents necessary for effective therapy will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medicants administered. Thus, 5 treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used *in vitro* may provide useful guidance in the amounts useful for *in situ* administration of these reagents. Animal testing of effective doses for treatment of particular disorders will 10 provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack 15 Publishing Co., Easton, Penn. Methods for administration are discussed therein and below, e.g., for oral, intravenous, intraperitoneal, or intramuscular administration, transdermal diffusion, and others. Pharmaceutically acceptable carriers will include water, saline, buffers, and other compounds 20 described, e.g., in the Merck Index, Merck & Co., Rahway, New Jersey. Dosage ranges would ordinarily be expected to be in amounts lower than 1 mM concentrations, typically less than about 10 μ M concentrations, usually less than about 100 nM, preferably less than about 10 pM (picomolar), and most 25 preferably less than about 1 fM (femtomolar), with an appropriate carrier. Slow release formulations, or a slow release apparatus will often be utilized for continuous administration.

CCF18 chemokine, fragments thereof, and antibodies to it 30 or its fragments, antagonists, and agonists, may be administered directly to the host to be treated or, depending on the size of the compounds, it may be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic 35 formulations may be administered in any conventional dosage formulation. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation. Formulations typically comprise

40

at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier should be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other 5 ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be 10 prepared by any methods well known in the art of pharmacy. See, e.g., Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn.; Avis, et al. 15 (eds.) (1993) Pharmaceutical Dosage Forms: Parenteral Medications Dekker, New York; Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Tablets Dekker, New York; and Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Disperse Systems Dekker, New York. The therapy of this 20 invention may be combined with or used in association with other chemotherapeutic or chemopreventive agents.

Both the naturally occurring and the recombinant form of the CCF18 chemokines of this invention are particularly useful in kits and assay methods which are capable of 25 screening compounds for binding activity to the proteins. Several methods of automating assays have been developed in recent years so as to permit screening of tens of thousands of compounds in a short period. See, e.g., Fodor, et al. (1991) Science 251:767, which describes means for testing of 30 binding affinity by a plurality of defined polymers synthesized on a solid substrate. The development of suitable assays can be greatly facilitated by the availability of large amounts of purified, soluble CCF18 chemokine as provided by this invention.

35 For example, antagonists can normally be found once the ligand has been structurally defined. Testing of potential ligand analogs is now possible upon the development of highly automated assay methods using physiologically responsive

41

cells. In particular, new agonists and antagonists will be discovered by using screening techniques described herein.

Viable cells could also be used to screen for the effects of drugs on CCF18 chemokine mediated functions, e.g., 5 second messenger levels, i.e., Ca^{++} ; inositol phosphate pool changes (see, e.g., Berridge (1993) *Nature* 361:315 or Billah and Anthes (1990) *Biochem. J.* 269:281); cellular morphology modification responses; phosphoinositide lipid turnover; an antiviral response, and others. Some detection methods allow 10 for elimination of a separation step, e.g., a proximity sensitive detection system. Calcium sensitive dyes will be useful for detecting Ca^{++} levels, with a fluorimeter or a fluorescence cell sorting apparatus.

Rational drug design may also be based upon structural 15 studies of the molecular shapes of the CCF18 chemokine and other effectors or analogs. Effectors may be other proteins which mediate other functions in response to ligand binding, or other proteins which normally interact with the receptor. One means for determining which sites interact with specific 20 other proteins is a physical structure determination, e.g., x-ray crystallography or 2 dimensional NMR techniques. These will provide guidance as to which amino acid residues form molecular contact regions. For a detailed description of protein structural determination, see, e.g., Blundell and 25 Johnson (1976) *Protein Crystallography*, Academic Press, New York.

Purified CCF18 chemokine can be coated directly onto plates for use in the aforementioned drug screening 30 techniques. However, non-neutralizing antibodies to these ligands can be used as capture antibodies to immobilize the respective ligand on the solid phase.

Kits

This invention also contemplates use of CCF18 chemokine 35 proteins, fragments thereof, peptides, and their fusion products in a variety of diagnostic kits and methods for detecting the presence of ligand, antibodies, or a CCF18 chemokine receptor. Typically the kit will have a

42

compartment containing either a defined CCF18 chemokine peptide or gene segment or a reagent which recognizes one or the other, e.g., antibodies.

A kit for determining the binding affinity of a test compound to a CCF18 chemokine would typically comprise a test compound; a labeled compound, for example an antibody having known binding affinity for the ligand; a source of CCF18 chemokine (naturally occurring or recombinant); and a means for separating bound from free labeled compound, such as a solid phase for immobilizing the ligand. Once compounds are screened, those having suitable binding affinity to the ligand can be evaluated in suitable biological assays, as are well known in the art, to determine whether they act as agonists or antagonists to the receptor. The availability of recombinant CCF18 chemokine polypeptides also provide well defined standards for calibrating such assays.

A preferred kit for determining the concentration of, for example, a CCF18 chemokine in a sample would typically comprise a labeled compound, e.g., antibody, having known binding affinity for the ligand, a source of ligand (naturally occurring or recombinant) and a means for separating the bound from free labeled compound, for example, a solid phase for immobilizing the CCF18 chemokine. Compartments containing reagents, and instructions, will normally be provided.

Antibodies, including antigen binding fragments, specific for the CCF18 chemokine or ligand fragments are useful in diagnostic applications to detect the presence of elevated levels of CCF18 chemokine and/or its fragments. Such diagnostic assays can employ lysates, live cells, fixed cells, immunofluorescence, cell cultures, body fluids, and further can involve the detection of antigens related to the ligand in serum, or the like. Diagnostic assays may be homogeneous (without a separation step between free reagent and antigen-ligand complex) or heterogeneous (with a separation step). Various commercial assays exist, such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), enzyme-multiplied

43

immunoassay technique (EMIT), substrate-labeled fluorescent immunoassay (SLFIA), and the like. For example, unlabeled antibodies can be employed by using a second antibody which is labeled and which recognizes the antibody to a CCF18 chemokine or to a particular fragment thereof. Similar assays have also been extensively discussed in the literature. See, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Press.

Anti-idiotypic antibodies may have similar use to
10 diagnose presence of antibodies against a CCF18 chemokine, as such may be diagnostic of various abnormal states. For example, overproduction of CCF18 chemokine may result in production of various immunological reactions which may be diagnostic of abnormal physiological states, particularly in
15 various inflammatory conditions.

Frequently, the reagents for diagnostic assays are supplied in kits, so as to optimize the sensitivity of the assay. For the subject invention, depending upon the nature of the assay, the protocol, and the label, either labeled or
20 unlabeled antibody or labeled CCF18 chemokine is provided. This is usually in conjunction with other additives, such as buffers, stabilizers, materials necessary for signal production such as substrates for enzymes, and the like. Preferably, the kit will also contain instructions for proper
25 use and disposal of the contents after use. Typically the kit has compartments for each useful reagent. Desirably, the reagents are provided as a dry lyophilized powder, where the reagents may be reconstituted in an aqueous medium providing appropriate concentrations of reagents for performing the
30 assay.

Any of the aforementioned constituents of the drug screening and the diagnostic assays may be used without modification or may be modified in a variety of ways. For example, labeling may be achieved by covalently or non-
35 covalently joining a moiety which directly or indirectly provides a detectable signal. In any of these assays, the ligand, test compound, CCF18 chemokine, or antibodies thereto can be labeled either directly or indirectly. Possibilities

44

for direct labeling include label groups: radiolabels such as 125I, enzymes (U.S. Pat. No. 3,645,090) such as peroxidase and alkaline phosphatase, and fluorescent labels (U.S. Pat. No. 3,940,475) capable of monitoring the change in 5 fluorescence intensity, wavelength shift, or fluorescence polarization. Possibilities for indirect labeling include biotinylation of one constituent followed by binding to avidin coupled to one of the above label groups.

There are also numerous methods of separating the bound 10 from the free ligand, or alternatively the bound from the free test compound. The CCF18 chemokine can be immobilized on various matrixes followed by washing. Suitable matrixes include plastic such as an ELISA plate, filters, and beads. Methods of immobilizing the CCF18 chemokine to a matrix 15 include, without limitation, direct adhesion to plastic, use of a capture antibody, chemical coupling, and biotin-avidin. The last step in this approach involves the precipitation of ligand/antibody complex by any of several methods including those utilizing, e.g., an organic solvent such as 20 polyethylene glycol or a salt such as ammonium sulfate. Other suitable separation techniques include, without limitation, the fluorescein antibody magnetizable particle method described in Rattle, et al. (1984) Clin. Chem. 30:1457, and the double antibody magnetic particle separation 25 as described in U.S. Pat. No. 4,659,678.

Methods for linking proteins or their fragments to the 30 various labels have been extensively reported in the literature and do not require detailed discussion here. Many of the techniques involve the use of activated carboxyl groups either through the use of carbodiimide or active 35 esters to form peptide bonds, the formation of thioethers by reaction of a mercapto group with an activated halogen such as chloroacetyl, or an activated olefin such as maleimide, for linkage, or the like. Fusion proteins will also find use in these applications.

Another diagnostic aspect of this invention involves use of oligonucleotide or polynucleotide sequences taken from the sequence of a CCF18 chemokine. These sequences can be used

45

as probes for detecting levels of the ligand message in samples from patients suspected of having an abnormal condition, e.g., an inflammatory or developmental problem. The preparation of both RNA and DNA nucleotide sequences, the 5 labeling of the sequences, and the preferred size of the sequences has received ample description and discussion in the literature. Normally an oligonucleotide probe should have at least about 14 nucleotides, usually at least about 18 nucleotides, and the polynucleotide probes may be up to 10 several kilobases. Various labels may be employed, most commonly radionuclides, particularly ^{32}P . However, other techniques may also be employed, such as using biotin modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or 15 antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescers, enzymes, or the like. Alternatively, antibodies may be employed which can recognize specific duplexes, including DNA duplexes, RNA duplexes, DNA-RNA hybrid duplexes, or DNA-protein duplexes. 20 The antibodies in turn may be labeled and the assay carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected. The use of probes to the novel anti-sense RNA may be carried out in any 25 conventional techniques such as nucleic acid hybridization, plus and minus screening, recombinational probing, hybrid released translation (HRT), and hybrid arrested translation (HART). This also includes amplification techniques such as polymerase chain reaction (PCR).

30 Diagnostic kits which also test for the qualitative or quantitative presence of other markers are also contemplated. Diagnosis or prognosis may depend on the combination of multiple indications used as markers. Thus, kits may test for combinations of markers. See, e.g., Viallet, et al. 35 (1989) Progress in Growth Factor Res. 1:89.

Receptor

46

Having isolated a ligand binding partner of a specific interaction, methods exist for isolating the counter-partner. See, Gearing, et al EMBO J. 8:3667. For example, means to 5 label a CCF18 chemokine without interfering with the binding to its receptor can be determined. For example, an affinity label can be fused to either the amino- or carboxy-terminus of the ligand. An expression library can be screened for specific binding of the CCF18 chemokine, e.g., by cell 10 sorting, or other screening to detect subpopulations which express such a binding component. See, e.g., Ho, et al. (1993) Proc. Nat'l Acad. Sci. 90:11267. Alternatively, a panning method may be used. See, e.g., Seed and Aruffo (1987) Proc. Nat'l. Acad. Sci. 84:3365.

15 Protein cross-linking techniques with label can be applied to isolate binding partners of a CCF18 chemokine. This would allow identification of protein which specifically interacts with a CCF18 chemokine, e.g., in a ligand-receptor like manner.

20 In another embodiment, a new receptor designated CC CKR3 was isolated. The sequences of the human construct and product are provided in Table 4. Similar means for making variants, at the nucleotide level or at the protein level, and making antibodies will be available as described above. 25 Many similar or related uses to the ligands will be applied to the receptors, as specific binding reagents. Many uses, including kits, will also be available through analogous techniques.

30 The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the invention to specific embodiments.

EXAMPLES

35 General Methods

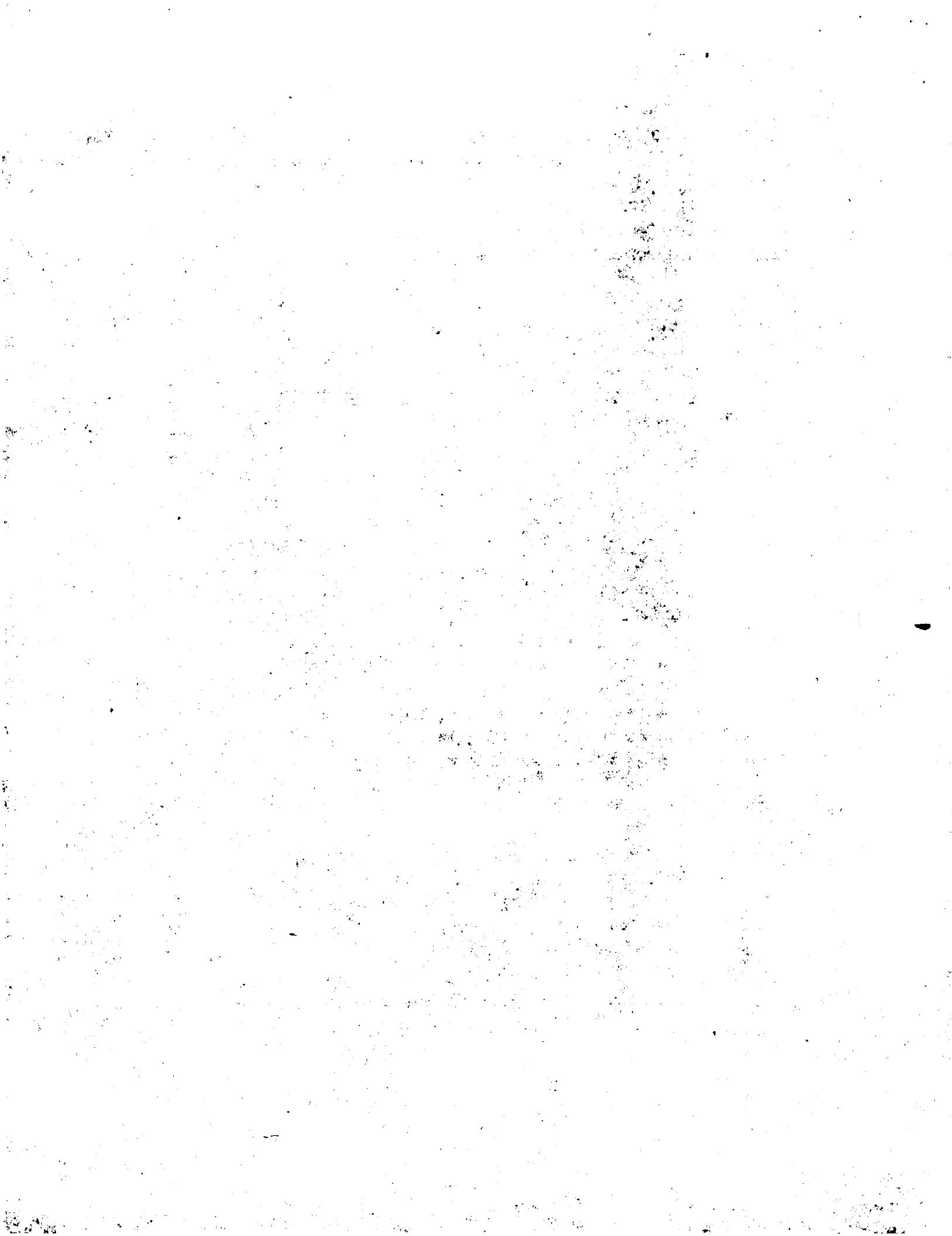
Some of the standard methods are described or referenced, e.g., in Maniatis, et al. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory,

Cold Spring Harbor Press; Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual, (2d ed.), vols 1-3, CSH Press, NY; Ausubel, et al., Biology, Greene Publishing Associates, Brooklyn, NY; or Ausubel, et al. (1987 and Supplements) 5 Current Protocols in Molecular Biology, Greene/Wiley, New York; Innis, et al. (eds.) (1990) PCR Protocols: A Guide to Methods and Applications Academic Press, N.Y. Methods for protein purification include such methods as ammonium sulfate precipitation, column chromatography, electrophoresis, 10 centrifugation, crystallization, and others. See, e.g., Ausubel, et al. (1987 and periodic supplements); Deutscher (1990) "Guide to Protein Purification" in Methods in Enzymology, vol. 182, and other volumes in this series; and manufacturer's literature on use of protein purification 15 products, e.g., Pharmacia, Piscataway, N.J., or Bio-Rad, Richmond, CA. Combination with recombinant techniques allow fusion to appropriate segments, e.g., to a FLAG sequence or an equivalent which can be fused via a protease-removable sequence. See, e.g., Hochuli (1989) Chemische Industrie 20 12:69; Hochuli (1990) "Purification of Recombinant Proteins with Metal Chelate Absorbent" in Setlow (ed.) Genetic Engineering, Principle and Methods 12:87, Plenum Press, N.Y.; and Crowe, et al. (1992) QIAexpress: The High Level Expression & Protein Purification System QUIAGEN, Inc., 25 Chatsworth, CA.

FACS analyses are described in Melamed, et al. (1990) Flow Cytometry and Sorting Wiley-Liss, Inc., New York, NY; Shapiro (1988) Practical Flow Cytometry Liss, New York, NY; and Robinson, et al. (1993) Handbook of Flow Cytometry 30 Methods Wiley-Liss, New York, NY.

Isolation and Sequencing of mouse and human CCF18 cDNA

BF-EGFR/EPORH is a Ba/F3 (mouse pre-B cell line; see Palacios, et al. (1985) Cell 41:727; and Palacios, et al. 35 (1984) Nature 309:126) transfected expressing a chimeric receptor between the extracellular domain of the epidermal growth factor (EGF) receptor and the cytoplasmic domain of the erythropoietin receptor and proliferates in response to



48

either IL-3 or EGF, see, e.g., Maruyama, et al. (1994) J. Biol. Chem. 269:5976. BF-EGFR/EPORH cells were maintained in RPMI 1640 containing 10% fetal calf serum (FCS) and IL-3 (100 units/ml). COS cells were cultured in DMEM containing 10% FCS. A cDNA library was made from EGF-stimulated BF-EGFR/EPORH cells, see, e.g., Yoshimura, et al. (1995) EMBO J. 14:2816. Isolation of poly(A)+ RNA, cDNA synthesis, and ligation into the expression vector, pME18S, were carried out as described Hara, et al. (1994) Blood 84:189.

10 The sequence of the CCF18 cDNA insert was determined by the Taq DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA) using oligonucleotide primers corresponding to the sequences of the vector and the cDNA insert. The open reading frame of mouse CCF18 cDNA encodes a 15 polypeptide of 123 amino acids including a putative signal sequence of 21 amino acid residues. The mature protein consists of about 102 amino acids with a calculated molecular weight of 11548 daltons. Computer searches with other C-C chemokine family members revealed significant homology at the 20 amino acid levels with MIP-1 β (67%), MIP-1 α (56%), JE (55%), RANTES (48%), lymphotactin (48%), and NSGA/Gro- α (43%).

A human fetal spleen cDNA library was purchased from Stratagene, though other libraries might also be successfully used. The library was screened by standard methods using 25 mouse CCF18 as a probe. See Hara, et al. (1995) J. Immunol. 155:5352. The isolate is described above and in Table 3.

Chromosomal location of mouse CCF18

Interspecific backcross progeny were generated by mating 30 (C57BL/6J x *M. spretus*) F1 females and C57BL/6J males as described in Copeland, et al. (1991) Trends Genet. 7:113. A total of 205 backcross mice were used to map the CCF18 gene, the Scya10 locus. DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot 35 transfer, and hybridization were performed essentially as described in Jenkins, et al. (1982) J. Virol. 43:26. All blots were prepared with Hybond-N+ nylon membrane (Amersham). The Scya10 probe, an ~1.1 kb EcoRI/NotI fragment of the CCF18

5 cDNA, was labeled with [α -³²P] dCTP using a nick translation labeling kit (Boehringer Mannheim); washing was done to a final stringency of 0.1 X SSCP, 0.1% SDS, 65° C. A 7.8 kb fragment was detected in BamHI-digested C57BL/6J DNA, while a 5.1 kb fragment was detected in *M. spretus* DNA. The presence or absence of the 5.1 kb *M. spretus*-specific BamHI fragment was followed in backcross mice.

10 A description of the probes and RFLPs for two of the loci linked to Scya10 including neurofibromatosis type 1 (Nf1) and myeloperoxidase (Mpo) has been reported previously in Buchberg, et al. (1988) Oncogene Res. 2:149; and Buchberg, et al. (1989) Genetics 122:153. The position of two other loci, Scyal and Scya2, used to position Scya10 on the Frederick interspecific backcross map, have not been reported 15 previously. The ~700 bp Scyal and ~700 bp Scya2 mouse cDNA probes were labeled with [α -³²P] dCTP by random priming; washing was done to a final stringency of 0.5 X SSCP, 0.1% SDS, 65° C. Two Scyal fragments, 10.5 and 0.5 kb in size, were detected in EcoRI-digested C57BL/6J DNA while 3.3 and 20 0.5 kb fragments were detected in EcoRI-digested *M. spretus* DNA. In addition, a 1.6 kb Scyal fragment was detected in PstI-digested C57BL/6J DNA while 2.6 and 1.6 kb fragments were detected in PstI-digested *M. spretus* DNA. The presence or absence of the 3.3 kb *M. spretus*-specific EcoRI fragment 25 and the 2.6 kb *M. spretus*-specific PstI fragment, which cosegregated, was followed in backcross mice. The two sets of data were combined to determine the map location of Scyal. Likewise, a major 5.5 kb Scya2 fragments was detected in TaqI-digested C57BL/6J DNA, while intensely hybridizing 7.4 30 and 6.3 kb Scya2 fragments were detected in *M. spretus* DNA. The presence or absence of the 7.4 and 6.3 kb *M. spretus*-specific TaqI fragments was followed in backcross mice. Backcross mice either inherited the 7.4 kb or 6.3 kb TaqI fragment but never both. This result indicates that the *M. spretus* mice used to generate the Frederick interspecific 35 backcross were still segregating for two Scya2 alleles. Recombination distances were calculated as described in Green (1981) "Linkage, recombination and mapping", Genetics and

Probability in Animal Breeding Experiments Oxford University Press, NY, 77, using the computer program SPRETUS MADNESS. Gene order was determined by minimizing the number of double and multiple recombination events across the chromosome.

5 The murine chromosomal location of CCF18 (designated gene symbol Scya10 for small inducible cytokine a10), determined by interspecific backcross analysis C57BL/6J and M. spretus DNAs were digested with several enzymes and analyzed by Southern blot hybridization for informative
10 restriction fragment length polymorphisms (RFLPs) using a mouse Scya10 cDNA probe. The 5.1 kb M. spretus-specific BamHI fragment was used to follow the segregation of the Scya10 locus in backcross DNAs. The mapping results indicated that Scya10 is located in the middle region of
15 mouse chromosome 11 tightly linked to two other members of the C-C chemokine family, Scya1 (Tca3) and Scya2 (Je). Although 55 mice were analyzed for five markers in the haplotype analysis, up to 134 mice were analyzed for some pairs of markers. Each locus was analyzed in pairwise
20 combinations for recombination frequencies using the additional data. The ratios of the total number of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci and the most likely gene order are: centromere-Nf1-5134-scya1-0/107-scya10-0/98-Scya2-
25 2/113-Mpo. The recombination frequencies [expressed as genetic distances in centiMorgans (cM) + the standard error] are centromere- Nf1 -3.7 + 1.6 - (Scya1, Scya10, Scya2) - 1.8 + 1.2 - Mpo. No recombinants were detected between Scya1 and Scya10 in 107 animals typed in common or between Scya10 and
30 Scya2 in 98 mice typed in common suggesting that the loci in each pair are within 2.2 and 3 cM of each other (95% confidence limit), respectively.

Three other C-C chemokine genes have recently been mapped in the vicinity of Scya10. These include MIP-1 α (Scya3), MIP-1 β (Scya4), and RANTES (Scya5). Scya5 did not recombine with Scya1, Scya2, and Scya10; Scya3 and Scya4 did not recombine with each other in 189 animals typed in common and the two genes map 0.6 +/- 0.6 cM distal of the Scya10

gene cluster. These results confirm and extend the earlier observations that all C-C chemokine genes map to the central region of mouse chromosome 11. See, Wilson, et al. (1990) J. Exp. Med. 171:1301; Berger, et al. (1993) DNA and Cell Biol. 12:839; and Danoff, et al. (1994) J. Immunol. 152:1182.

Finally, the middle region of mouse chromosome 11 shares a region of homology with human chromosome 17q. In particular, SCYA1 and SCYA2 have been mapped to human 17q12 and 17q11.2-q12, respectively. This suggests that the human homolog of CCF18 (the Scya10 locus) should map to 17q11-q12, as well.

Assays for chemotactic activity

To obtain the CCF18 protein, COS cells were transfected with a plasmid carrying the CCF18 cDNA by electroporation. See, Hara, et al. (1992) EMBO J. 10:1875. Three days after transfection, a culture supernatant was collected and subjected to bioassays. As a mock control, a plasmid carrying the luciferase cDNA was used. See, de Wet, et al. (1987) Mol. Cell. Biol. 7:725. Recombinant murine MIP-1 α was purchased from R&D Systems (Minneapolis, MN).

Lymphocyte migration assays were performed as previously described in Bacon, et al. (1988) Br. J. Pharmacol. 95:966 with the modification that optimal migration was observed only after 2 hours incubation of the assay. Two murine Th2 T cell clones, CDC-25 (see Tony, et al. (1985) J. Exp. Med. 161:223) and HDK-1 (see Cherwinski, et al. (1987) J. Exp. Med. 166:1229) were kindly provided by R. Coffman and A. O'Garra (DNAX, Palo Alto, CA), respectively. Ca²⁺ flux upon chemokine stimulation was measured according to the published procedure described in Bacon, et al. (1995) J. Immunol. 154:3654.

Maximal numbers of migrating cells in response to MIP-1 α occurred at a concentration of 10⁻⁸ M, in agreement with original reports for CD4+ populations of human T cells. See Schall (1993) J. Exp. Med. 177:1821. The murine T cell clones responded to both CCF18 and MIP-1 α with similar maximal cell numbers, giving a characteristic bell shaped

dose-response curve. A maximum number of the T lymphocytes migrated in the presence of a 500-1000 fold dilution of the CCF18 containing supernatant. At higher concentrations of CCF18, the cells adhered to the upper surface of the filter, 5 accounting for the lower number of cells recorded on the undersurface. In all experiments the same concentration range of mock supernatant was tested but failed to have any effect on the cells. Assay for chemotactic versus chemokinetic activity demonstrated that CCF18 elicited 10 chemotactic migration.

After stimulation with C-C chemokines, lymphocytes generally show a measurable intracellular Ca^{2+} flux. This was also the case with CCF18. Both MIP-1 α and CCF18 were capable of inducing immediate transients of calcium 15 mobilization. However, MIP-1 α completely desensitized the cells to subsequent administration of CCF18. Prior administration of CCF18 only partially desensitized the response to subsequent administration of MIP-1 α as evidenced by the smaller calcium flux profile.

20 The levels of CCF18 used in these assays were similar to those used for the chemotaxis assays (1/1000 dilution of conditioned supernatants). In all instances, the control COS supernatants (mock transfected) failed to elicit any calcium mobilization response.

25

Northern blot analysis of mouse CCF18

RNA blot and hybridization was performed according to the standard method in Maniatis, et al. (1982) Molecular Cloning: A Laboratory Manual Cold Spring Harbor Laboratory 30 Press, Cold Spring Harbor, NY, by using an 1.1 kb EcoRI/NotI CCF18 cDNA fragment as a probe. To verify the amount of RNA loaded in each lane, the membrane was reprobed with WAF1 cDNA, see El-Deiry, et al. (1993) Cell 75:817, which was obtained during screening of the cDNA library in this study, 35 or glyceraldehyde 3-phosphate dehydrogenase (G3PDH) cDNA (Clontech, Palo Alto CA).

The CCF18 mRNA (approximately 1.4 kb in size for the major band) was constitutively expressed at a relatively high

level in a macrophage cell line, P388D1, and an IL-3-dependent myeloid cell line, 32D. A small amount of CCF18 mRNA was also detected in an IL-3-dependent mast cell line, MC/9, a preB cell line, Y16, and a fibroblastic cell line, NIH3T3. However, a T-cell line, CTLL-2, and the proB line, Ba/F3, do not express CCF18 mRNA. In addition, the expression level of CCF18 mRNA in Ba/F3 cells varied from clone to clone. In BF-EGFR/EPORH, CCF18 mRNA was detected at highest and constitutively expressed without cytokine stimulation, whereas in other Ba/F3 transfectants, 626 and BF-EGFR, the amount of the CCF18 transcript was low and appeared to be cytokine-inducible in 626 cells. A cDNA encoding p21 WAF1, whose mRNA expression is enhanced by cytokine stimulation, was used to verify the RNA amount and cytokine-response in those cell lines. Thus, it is not clear how the production of CCF18 is regulated because the expression of the C-C chemokines appears to be differentially regulated by a variety of stimuli. See, Orlofsky (1994) *J. Immunol.* 152:5084.

20

Isolation of human chemokine receptor, CC CKR3, cDNA

The CC CKR3 cDNA was isolated using the procedure described in Neote, et al. (1993) *Cell* 72:415-425. Two degenerate PCR primers were designed using sequences from the conserved regions of known chemokine receptors. These primers were used to amplify specific regions of human genomic DNA.

30

Degenerate PCR primers

35

1. DRYLAIIVHAdeg(+)
5'-GAT CGI TAG CTI GCI ATI; GTI CA(T/C) GC -3'
2. TM7deg(-)α
5'-CG GAA III (C/A)TC IC(G/C) IAC (G/A)AA IGC(G/A)TA -3'

I represents deoxyInosine.

40

Of the DNA sequences identified by this method, one represented a portion of the CC CKR3 gene. This DNA fragment was then used to screen a Th0 activated T cell cDNA library

provided by T. McClanahan (DNAX, Palo Alto CA), in order to obtain the full length cDNA.

Generation of antibodies to CCF18 and CC CRK3

5 The purified protein or defined peptides are useful for generating antibodies by standard methods, as described above. Synthetic peptides or purified protein are presented to an immune system to generate monoclonal or polyclonal antibodies. See, e.g., Coligan (1991) Current Protocols in
10 Immunology Wiley/Greene; and Harlow and Lane (1989) Antibodies: A Laboratory Manual Cold Spring Harbor Press. The binding reagent is either labeled as described above, e.g., fluorescence or otherwise, or immobilized to a substrate for panning methods.

15 The binding composition is used for screening of an expression library made from a cell line which expresses a CCF18 chemokine. Standard staining techniques are used to detect or sort intracellular or surface expressed ligand, or surface expressing transformed cells are screened by panning.

20 Screening of intracellular expression is performed by various staining or immunofluorescence procedures. See also, e.g., McMahan, et al. (1991) EMBO J. 10:2821.

25 For example, on day 0, precoat 2-chamber permanox slides with 1 ml per chamber of fibronectin, 10 ng/ml in PBS, for 30 min at room temperature. Rinse once with PBS. Then plate COS cells at $2-3 \times 10^5$ cells per chamber in 1.5 ml of growth media. Incubate overnight at 37° C.

30 On day 1 for each sample, prepare 0.5 ml of a solution of 66 μ g/ml DEAE-dextran, 66 μ M chloroquine, and 4 μ g DNA in serum free DME. For each set, a positive control is prepared, e.g., of huIL-10-FLAG cDNA at 1 and 1/200 dilution, and a negative mock. Rinse cells with serum free DME. Add the DNA solution and incubate 5 hr at 37° C. Remove the medium and add 0.5 ml 10% DMSO in DME for 2.5 min. Remove and wash once with DME. Add 1.5 ml growth medium and incubate overnight.

35 On day 2, change the medium. On days 3 or 4, the cells are fixed and stained. Rinse the cells twice with Hank's

Buffered Saline Solution (HBSS) and fix in 4% paraformaldehyde (PFA)/glucose for 5 min. Wash 3X with HBSS. The slides may be stored at -80° C after all liquid is removed. For each chamber, 0.5 ml incubations are performed 5 as follows. Add HBSS/saponin(0.1%) with 32 μ l/ml of 1M NaN₃ for 20 min. Cells are then washed with HBSS/saponin 1X. Add antibody complex to cells and incubate for 30 min. Wash cells twice with HBSS/saponin. Add second antibody, e.g., Vector anti-mouse antibody, at 1/200 dilution, and incubate 10 for 30 min. Prepare ELISA solution, e.g., Vector Elite ABC horseradish peroxidase solution, and preincubate for 30 min. Use, e.g., 1 drop of solution A (avidin) and 1 drop solution B (biotin) per 2.5 ml HBSS/saponin. Wash cells twice with HBSS/saponin. Add ABC HRP solution and incubate for 30 min. 15 Wash cells twice with HBSS, second wash for 2 min, which closes cells. Then add Vector diaminobenzoic acid (DAB) for 5 to 10 min. Use 2 drops of buffer plus 4 drops DAB plus 2 drops of H₂O₂ per 5 ml of glass distilled water. Carefully remove chamber and rinse slide in water. Air dry for a few 20 minutes, then add 1 drop of Crystal Mount and a cover slip. Bake for 5 min at 85-90° C.

Alternatively, the binding compositions are used to-affinity purify or sort out cells expressing the ligand. See, e.g., Sambrook, et al. or Ausubel, et al.

25 Many modification and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of 30 the appended claims, along with the full scope of the equivalents to which such claims are entitled.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(i) APPLICANT: SCHERING CORPORATION

10

(ii) INVENTORS: Dairaghi, Daniel J.
Hara, Takahiko
Miyajima, Atsushi
Schall, Thomas J.
Wang, Wei
Yoshimura, Akihiko

15

(iii) TITLE OF INVENTION: MAMMALIAN CHEMOKINE REAGENTS

(iv) NUMBER OF SEQUENCES: 9

(v) CORRESPONDENCE ADDRESS:

20

(A) ADDRESSEE: Schering Corporation
(B) STREET: 2000 Galloping Hill Road
(C) CITY: Kenilworth
(D) STATE: New Jersey
(E) COUNTRY: USA
25 (F) ZIP: 07033

(v) COMPUTER READABLE FORM:

30

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: Apple Macintosh
(C) OPERATING SYSTEM: Macintosh 7.5.3
(D) SOFTWARE: Microsoft Word 5.1a

35

(vi) PRIORITY APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/567,882
(B) FILING DATE: 08-DEC-1995
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

40

(A) NAME: Cynthia L. Foulke
(B) REGISTRATION NUMBER: 32,364
(C) REFERENCE/DOCKET NUMBER: DX0506

45

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 908-298-2987
(B) TELEFAX: 908-298-5388

(2) INFORMATION FOR SEQ ID NO:1:

50

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1165 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 53.421

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

5	GCCTCGTGCC GAATTCGGCA CGAGGGGCCA GCTGGGTCTG CCCACTAAGA AG ATG Met 1	55
10	AAG CCT TTT CAT ACT GCC CTC TCC TTC CTC ATT CTT ACA ACT GCT CTT Lys Pro Phe His Thr Ala Leu Ser Phe Leu Ile Leu Thr Thr Ala Leu 5 10 15	103
15	GGA ATC TGG GCC CAG ATC ACA CAT GCA ACA GAG ACA AAA GAA GTC CAG Gly Ile Trp Ala Gln Ile Thr His Ala Thr Glu Thr Lys Glu Val Gln 20 25 30	151
20	AGC AGT CTG AAG GCA CAG CAA GGG CTT GAA ATT GAA ATG TTT CAC ATG Ser Ser Leu Lys Ala Gln Gln Gly Leu Glu Ile Glu Met Phe His Met 35 40 45	199
25	GGC TTT CAA GAC TCT TCA GAT TGC TGC CTG TCC TAT AAC TCA CGG ATT Gly Phe Gln Asp Ser Ser Asp Cys Cys Leu Ser Tyr Asn Ser Arg Ile 50 55 60 65	247
30	CAG TGT TCA AGA TTT ATA GGT TAT TTT CCC ACC AGT GGT GGG TGT ACC Gln Cys Ser Arg Phe Ile Gly Tyr Phe Pro Thr Ser Gly Gly Cys Thr 70 75 80	295
35	AGG CCG GGC ATC ATC TTT ATC AGC AAG AGG GGG TTC CAG GTC TGT GCC Arg Pro Gly Ile Ile Phe Ile Ser Lys Arg Gly Phe Gln Val Cys Ala 85 90 95	343
40	AAC CCC AGT GAT CGG AGA GTT CAG AGA TGC ATT GAA AGA TTG GAG CAA Asn Pro Ser Asp Arg Arg Val Gln Arg Cys Ile Glu Arg Leu Glu Gln 100 105 110	391
45	AAC TCA CAA CCA CGG ACC TAC AAA CAA TAACATTTGC TTGAAGAGAA Asn Ser Gln Pro Arg Thr Tyr Lys Gin 115 120	438
50	GGGTGTGAAC TGCCAGCTAC TTTCTTTGGT CTTCCCCAGT GACCACCTAA GCGGCTCTAA GTGTTTATTT TTATAGGTAT ATAAACATTT TTTTTCTGT TCCACTTTAA AGTGGCATAT CTGGCTTTGT CACAGAGGGG AAACCTGTCT GTGCCAACCC CAGTCATCTG AAAACTCAGA TGCCTGCCGA AGGTCTGAAG CTGACTCAAT GACTACACAT AATATTTGAT TGAGATAAAT	498 558 618 678
55	GGCAAGGTC TGGAGAGATG GCTTGGTGGT TAAGAGCACC TGCTGCTCTT CCAGAGGACC TGGGTTCAAT TCCCACCTAG ATGGCAGCTC AAACTATCTA TAATTCCAAT TCCAAAGAAA ACTGATGCCC TATTTTGCCC TTTAGTTAGT AGTATTTACA GTATTCTTAA TAAATTCAACC TTGACATGAC CATCTTGAGC TACAGCCATC CTAACCGCCT CAGAACACT CAAGTTCTTC	738 798 858 918
60	CACTCGGTTT CCCAGCGGAT TATAAGTGGG TAAACTGTGA GAGTGGTCTG TGGGACTTTG GAATGTGTCT GGTTCTGATA GTCACATTATG GCAACCCGGG TACATTCAAC TAGGATGAAA	978 1038
65	TAAATTCTGC CTTAGCCCCAG TAGTATGTCT GTGTTGTAA GGACCCAGCT GATTTCCCCA	1098

CCACCCGTCC ATCAGTAAGC CACTAATAAA GTGCATCTAT GCAGCCAAAA AAAAAAAA 1158
 5 AAAAAAAA 1165

(2) INFORMATION FOR SEQ ID NO:2:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 122 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Lys Pro Phe His Thr Ala Leu Ser Phe Leu Ile Leu Thr Thr Ala
 1 5 10 15
 20 Leu Gly Ile Trp Ala Gln Ile Thr His Ala Thr Glu Thr Lys Glu Val
 20 25 30
 25 Gln Ser Ser Leu Lys Ala Gln Gln Gly Leu Glu Ile Glu Met Phe His
 35 40 45
 30 Met Gly Phe Gln Asp Ser Ser Asp Cys Cys Leu Ser Tyr Asn Ser Arg
 50 55 60
 35 Ile Gln Cys Ser Arg Phe Ile Gly Tyr Phe Pro Thr Ser Gly Gly Cys
 65 70 75 80
 40 Thr Arg Pro Gly Ile Ile Phe Ile Ser Lys Arg Gly Phe Gln Val Cys
 85 90 95
 Ala Asn Pro Ser Asp Arg Arg Val Gln Arg Cys Ile Glu Arg Leu Glu
 100 105 110
 45 Gln Asn Ser Gln Pro Arg Thr Tyr Lys Gln
 115 120

(2) INFORMATION FOR SEQ ID NO:3:

45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 348 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 1..342

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG AAG GTC TCC GTG GCT GCC CTC TCC TGC CTC ATG CTT GTT GCT GTC
 Met Lys Val Ser Val Ala Ala Leu Ser Cys Leu Met Leu Val Ala Val
 60 1 5 10 15 48

	CTT GGA TCC CAG GCC CAG TTC ATA AAT GAT GCA GAG ACA GAG TTA ATG Leu Gly Ser Gln Ala Gln Phe Ile Asn Asp Ala Glu Thr Glu Leu Met 20 25 30	96
5	ATG TCA AAG CTT CCA CTG GAA AAT CCA GTA GTT CTG AAC AGC AGC TTT CAC Met Ser Lys Leu Pro Leu Glu Asn Pro Val Val Leu Asn Ser Phe His 35 40 45	144
10	TTT GCT GCT GAC TGC TGC ACC TCC TAC ATC TCA CAA AGC ATC CCG TGT Phe Ala Ala Asp Cys Cys Thr Ser Tyr Ile Ser Gln Ser Ile Pro Cys 50 55 60	192
15	TCA CTC ATG AAA AGT TAT TTT GAA ACG AGC AGC GAG TGC TCC AAG CCA Ser Leu Met Lys Ser Tyr Phe Glu Thr Ser Ser Glu Cys Ser Lys Pro 65 70 75 80	240
20	GGT GTC ATA TTC CTC ACC AAG AAG GGG CGG CAA GTC TGT GCC AAA CCC Gly Val Ile Phe Leu Thr Lys Lys Gly Arg Gln Val Cys Ala Lys Pro 85 90 95	288
25	AGT GGT CCG GGA GTT CAG GAT TGC ATG AAA AAG CTG AAG CCC TAC TCA Ser Gly Pro Gly Val Gln Asp Cys Met Lys Lys Leu Lys Pro Tyr Ser 100 105 110	336
	ATA TAATAATAA Ile	348

30 (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 113 amino acids
 - (B) TYPE: amino acid
 - (C) TOPOLOGY: linear
 - (D) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

40	Met Lys Val Ser Val Ala Ala Leu Ser Cys Leu Met Leu Val Ala Val 1 5 10 15
45	Leu Gly Ser Gln Ala Gln Phe Ile Asn Asp Ala Glu Thr Glu Leu Met 20 25 30
50	Met Ser Lys Leu Pro Leu Glu Asn Pro Val Val Leu Asn Ser Phe His 35 40 45
55	Phe Ala Ala Asp Cys Cys Thr Ser Tyr Ile Ser Gln Ser Ile Pro Cys 50 55 60
60	Ser Leu Met Lys Ser Tyr Phe Glu Thr Ser Ser Glu Cys Ser Lys Pro 65 70 75 80
	Gly Val Ile Phe Leu Thr Lys Lys Gly Arg Gln Val Cys Ala Lys Pro 85 90 95
	Ser Gly Pro Gly Val Gln Asp Cys Met Lys Lys Leu Lys Pro Tyr Ser 100 105 110

Ile

5 (2) INFORMATION FOR SEQ ID NO:5:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 769 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

	CAGTGAGCCC	AGGAGTCCTC	GGCCAGCCCT	GCCTGCCAC	CAGGAGGATG	AAGGTCTCCG	60
	TGGCTGCCCT	CTCCTGCCTC	ATGCTGTG	CTGTCCTTGG	ATCCCAGGCC	CAGTTCATAA	120
20	ATGATGCAGA	GACAGAGTTA	ATGATGCAA	AGCTTCCACT	GGAAAATCCA	GTAGTTCTGA	180
	ACAGCTTTCA	CTTGTGCTGCT	GACTGCTGCA	CCTCCTACAT	CTCACAAAGC	ATCCCGTGT	240
25	CACTCATGAA	AAGTTATTTT	GAAACGAGCA	GCGAGTGCTC	CAAGCCAGGT	GTCATATTCC	300
	TCACCAAGAA	GGGGNGGCAA	GTCTGTGCCA	AACCCAGTGG	TCCGGGAGTT	CAGGATTGCA	360
	TGAAAAAGCT	GAAGCCCTAC	TCAATATAAT	AATAAAGAGA	CAAAAGAGGN	CAGCCACCCA	420
30	CCTCCAACAC	CTCCTGTGAG	TTTCITGGTC	TGAAATACTT	AAAAAAATATA	TATATTGTTG	480
	TGCTGGTAA	TGAAAGTAAT	GCATCTAATA	AAGAGTATTC	AATTTTTAA	AAAAAAAAAA	540
35	AAAAAAAGAA	AAAAAAAGAA	AAAAAANCT	CGGGGGGGGG	CCCGGTCCCC	AATCCGCCCT	600
	NTGGGGAGTC	GTTCNAAAAT	CCACTGGCCG	CCGTTTTAAA	ACGTNGGGAT	TGGGAAAACC	660
	CNGGGTTNC	CCAACCTTANT	CNCCTNGGAG	AANATCCCC	TTTCCGCCAG	TTGGGGTTAA	720
40	TAGGAAAGGA	GGCCCGNACC	GATCCGCCCT	TCCCCAAAAG	GNGGGGAGN		769

45 (2) INFORMATION FOR SEQ ID NO:6:

50 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1071 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..1071

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATG	ACA	ACC	TCA	CTA	GAT	ACA	GTT	GAG	ACC	TTT	GGT	ACC	ACA	TCC	TAC	48
Met	Thr	Thr	Ser	Leu	Asp	Thr	Val	Glu	Thr	Phe	Gly	Thr	Thr	Ser	Tyr	
60	1	5							10					15		

61

	TAT GAT GAC GTG GGC CTG CTC TGT GAA AAA GCT GAT ACC AGA GCA CTG Tyr Asp Asp Val Gly Leu Leu Cys Glu Lys Ala Asp Thr Arg Ala Leu 20 25 30	96
5	ATG GCC CAG TTT GTG CCC CCG CTG TAC TCC CTG GTG TTC ACT GTG GGC Met Ala Gln Phe Val Pro Pro Leu Tyr Ser Leu Val Phe Thr Val Gly 35 40 45	144
10	CTC TTG GGC AAT GTG GTG GTG ATG ATC CTC ATA AAA TAC AGG AGG Leu Leu Gly Asn Val Val Val Met Ile Leu Ile Lys Tyr Arg Arg 50 55 60	192
15	CTC CGA ATT ATG ACC AAC ATC TAC CTG CTC AAC CTG GCC ATT TCG GAC Leu Arg Ile Met Thr Asn Ile Tyr Leu Leu Asn Leu Ala Ile Ser Asp 65 70 75 80	240
	CTG CTC TTC CTC GTC ACC CTT CCA TTC TGG ATC CAC TAT GTC AGG GGG Leu Leu Phe Leu Val Thr Leu Pro Phe Trp Ile His Tyr Val Arg Gly 85 90 95	288
20	CAT AAC TGG GTT TTT GGC CAT GGC ATG TGT AAG CTC CTC TCA GGG TTT His Asn Trp Val Phe Gly His Gly Met Cys Lys Leu Leu Ser Gly Phe 100 105 110	336
25	TAT CAC ACA GGC TTG TAC AGC GAG ATC TTT TTC ATA ATC CTG CTG ACA Tyr His Thr Gly Leu Tyr Ser Glu Ile Phe Phe Ile Ile Leu Leu Thr 115 120 125	384
30	ATC GAC AGG TAC CTG GCC ATT GTC CAT GCT GTG TTT GCC CTT CGA GCC Ile Asp Arg Tyr Leu Ala Ile Val His Ala Val Phe Ala Leu Arg Ala 130 135 140	432
35	CGG ACT GTC ACT TTT GGT GTC ATC ACC AGC ATC GTC ACC TGG GGC CTC Arg Thr Val Thr Phe Gly Val Ile Thr Ser Ile Val Thr Trp Gly Leu 145 150 155 160	480
	GCA GTG CTA GCA GCT CTT CCT GAA TTT ATC TTC TAT GAG ACT GAA GAG Ala Val Leu Ala Ala Leu Pro Glu Phe Ile Phe Tyr Glu Thr Glu Glu 165 170 175	528
40	TTG TTT GAA GAG ACT CTT TGC AGT GCT CTT TAC CCA GAG GAT ACA GTA Leu Phe Glu Glu Thr Leu Cys Ser Ala Leu Tyr Pro Glu Asp Thr Val 180 185 190	576
45	TAT AGC TGG AGG CAT TTC CAC ACT CTG AGA ATG ACC ATC TTC TGT CTC Tyr Ser Trp Arg His Phe His Thr Leu Arg Met Thr Ile Phe Cys Leu 195 200 205	624
50	GTT CTC CCT CTG CTC GTT ATG GCC ATC TGC TAC ACA GGA ATC ATC AAA Val Leu Pro Leu Leu Val Met Ala Ile Cys Tyr Thr Gly Ile Ile Lys 210 215 220	672
	ACG CTG CTG AGG TGC CCC AGT AAA AAA AAG TAC AAG GCC ATC CGG CTC Thr Leu Leu Arg Cys Pro Ser Lys Lys Lys Tyr Lys Ala Ile Arg Leu 225 230 235 240	720
	ATT TTT GTC ATC ATG GCG GTG TTT TTC ATT TTC TGG ACA CCC TAC AAT Ile Phe Val Ile Met Ala Val Phe Phe Ile Phe Trp Thr Pro Tyr Asn 245 250 255	768
60	GTG GCT ATC CTT CTC TCT TCC TAT CAA TCC ATC TTA TTT GGA AAT GAC Val Ala Ile Leu Leu Ser Ser Tyr Gln Ser Ile Leu Phe Gly Asn Asp 260 265 270	816

62

5	TGT GAG CGG AGC AAG CAT CTG GAC CTG GAC ATG CTG GTG ACA GAG GTG Cys Glu Arg Ser Lys His Leu Asp Leu Asp Met Leu Val Thr Glu Val 275 280 285	864
10	ATC GCC TAC TCC CAC TGG TGC TGC CTC AAT CCC CTC ATC TAC GCC TTT Ile Ala Tyr Ser His Trp Cys Cys Leu Asn Pro Leu Ile Tyr Ala Phe 290 295 300	912
15	GTT GGA GAG AGG TTC CGG AAG TAC CTG CGC CAC TTC TTC CAC AGG CAC Val Gly Glu Arg Phe Arg Lys Tyr Leu Arg His Phe Phe His Arg His 305 310 315 320	960
20	TTG CTC ATG CAC CTG GGC AGA TAC ATC CCA TTC CTT CCT AGT GAG AAG Leu Leu Met His Leu Gly Arg Tyr Ile Pro Phe Leu Pro Ser Glu Lys 325 330 335	1008
25	CTG GAA AGA ACC AGC TCT GTC TCT CCA TCC ACA GGA GAG CCG GAA CTC Leu Glu Arg Thr Ser Ser Val Ser Pro Ser Thr Gly Glu Pro Glu Leu 340 345 350	1056
	TCT ATT GTG TTT TAG Ser Ile Val Phe 355	1071

(2) INFORMATION FOR SEQ ID NO:7:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 356 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: protein

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Thr Thr Ser Leu Asp Thr Val Glu Thr Phe Gly Thr Thr Ser Tyr 1 5 10 15
40 Tyr Asp Asp Val Gly Leu Leu Cys Glu Lys Ala Asp Thr Arg Ala Leu 20 25 30
45 Met Ala Gln Phe Val Pro Pro Leu Tyr Ser Leu Val Phe Thr Val Gly 35 40 45
Leu Leu Gly Asn Val Val Val Met Ile Leu Ile Lys Tyr Arg Arg 50 55 60
55 Leu Arg Ile Met Thr Asn Ile Tyr Leu Leu Asn Leu Ala Ile Ser Asp 65 70 75 80
60 Leu Leu Phe Leu Val Thr Leu Pro Phe Trp Ile His Tyr Val Arg Gly -85 90 95
His Asn Trp Val Phe Gly His Gly Met Cys Lys Leu Leu Ser Gly Phe 100 105 110
65 Tyr His Thr Gly Leu Tyr Ser Glu Ile Phe Phe Ile Ile Leu Leu Thr 115 120 125

63

Ile Asp Arg Tyr Leu Ala Ile Val His Ala Val Phe Ala Leu Arg Ala
 130 135 140

5 Arg Thr Val Thr Phe Gly Val Ile Thr Ser Ile Val Thr Trp Gly Leu
 145 150 155 160

Ala Val Leu Ala Ala Leu Pro Glu Phe Ile Phe Tyr Glu Thr Glu Glu
 165 170 175

10 Leu Phe Glu Glu Thr Leu Cys Ser Ala Leu Tyr Pro Glu Asp Thr Val
 180 185 190

Tyr Ser Trp Arg His Phe His Thr Leu Arg Met Thr Ile Phe Cys Leu
 195 200 205

15 Val Leu Pro Leu Leu Val Met Ala Ile Cys Tyr Thr Gly Ile Ile Lys
 210 215 220

Thr Leu Leu Arg Cys Pro Ser Lys Lys Tyr Lys Ala Ile Arg Leu
 225 230 235 240

Ile Phe Val Ile Met Ala Val Phe Phe Ile Phe Trp Thr Pro Tyr Asn
 245 250 255

25 Val Ala Ile Leu Leu Ser Ser Tyr Gln Ser Ile Leu Phe Gly Asn Asp
 260 265 270

Cys Glu Arg Ser Lys His Leu Asp Leu Asp Met Leu Val Thr Glu Val
 275 280 285

30 Ile Ala Tyr Ser His Trp Cys Cys Leu Asn Pro Leu Ile Tyr Ala Phe
 290 295 300

35 Val Gly Glu Arg Phe Arg Lys Tyr Leu Arg His Phe Phe His Arg His
 305 310 315 320

Leu Leu Met His Leu Gly Arg Tyr Ile Pro Phe Leu Pro Ser Glu Lys
 325 330 335

40 Leu Glu Arg Thr Ser Ser Val Ser Pro Ser Thr Gly Glu Pro Glu Leu
 340 345 350

Ser Ile Val Phe
 355

45

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
 50 (A) LENGTH: 26 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
 60 (A) NAME/KEY: misc_feature
 (B) LOCATION: 1
 (D) OTHER INFORMATION: /product= "N=deoxyInosine"

64

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GATCGNTAGC TNGCNATNGT NCAYGC

26

5 (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- 10 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

15 (ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1
- (D) OTHER INFORMATION: /product= "N=deoxyInosine"

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGGAANNNMT CNCASNACRAA NGCRTA

26

WHAT IS CLAIMED IS:

1. A composition selected from the group consisting of:
 - 5 a) a substantially pure CCF18 chemokine;
 - b) a fusion protein comprising CCF18 chemokine sequence;
 - c) an antibody specific for binding to a CCF18 chemokine; and
 - 10 d) a nucleic acid encoding a CCF18 chemokine or fusion protein thereof.
2. The CCF18 chemokine of Claim 1, wherein said chemokine:
 - 15 a) is from a warm blooded animal selected from the group of birds and mammals, including a mouse or human;
 - b) comprises a sequence defined by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4;
 - c) exhibits a post-translational modification pattern
 - 20 distinct from natural CCF18 chemokine; or
 - d) exhibits the features disclosed in Table 2.
3. A composition comprising said chemokine of Claim 2, and a pharmaceutically acceptable carrier.
- 25
4. The fusion protein of Claim 1, comprising:
 - a) a sequence defined by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4; and/or
 - b) a sequence of another cytokine or chemokine.
- 30
5. The antibody of Claim 1, wherein:
 - a) said CCF18 chemokine is a mammalian protein, including a mouse or human;
 - b) said antibody is raised against a peptide sequence
 - 35 defined by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4;
 - c) said antibody is a monoclonal antibody; or
 - d) said antibody is labeled.

66

6. A nucleic acid of Claim 1, wherein:

- a) said chemokine is from a warm blooded animal selected from the group of birds and mammals, including a mouse or human;
- 5 b) said nucleic acid comprises a sequence of Table 1 or 3;
- c) said nucleic acid is an expression vector; or
- d) said nucleic acid comprises a deoxyribonucleic acid nucleotide.

10

7. A kit comprising:

- a) a substantially pure CCF18 chemokine of Claim 1, or fragment thereof;
- b) an antibody which specifically binds a mammalian CCF18 chemokine; or
- 15 c) a nucleic acid encoding a CCF18 chemokine or peptide.

8. The kit of Claim 7 capable of making a qualitative 20 or quantitative analysis.

9. A method of modulating physiology or development of a cell comprising contacting said cell with an agonist or antagonist of a mammalian CCF18 chemokine.

25

10. The method of Claim 9, wherein said antagonist is an antibody against a mammalian CCF18 chemokine.

30

11. The method of Claim 9, wherein said cell is a hematopoietic cell, including a lymphoid cell; a placenta cell; a gonad cell; or a neural cell, including neuronal or non-neuronal cells.

35

12. The method of Claim 9, wherein said physiology is selected from:

- a) a cellular calcium flux;
- b) a chemoattractant response;
- c) cellular morphology modification responses;

67

- d) phosphoinositide lipid turnover; or
- e) an antiviral response.

13. A composition selected from the group consisting
5 of:

- a) a substantially pure CCKR3 chemokine receptor;
- b) a fusion protein comprising CCKR3 chemokine receptor sequence;
- c) an antibody specific for binding to a CCKR3 chemokine receptor; and
- 10 d) a nucleic acid encoding a CCKR3 chemokine receptor or fusion protein thereof.

14. The CCKR3 chemokine receptor of Claim 13, wherein
15 said receptor:

- a) is from a warm blooded animal selected from the group of birds and mammals, including a mouse or human;
- b) comprises a sequence of Table 4; or
- c) exhibits a post-translational modification pattern
20 distinct from natural CCKR3 chemokine receptor.

15. The fusion protein of Claim 13, comprising:

- a) a sequence defined by SEQ ID NO:5 and SEQ ID NO:6; and/or
- 25 b) sequence of another receptor for a chemokine.

16. The antibody of Claim 13, wherein:

- a) said CCKR3 chemokine receptor is a mammalian protein, including a mouse or human;
- 30 b) said antibody is raised against a peptide sequence defined by SEQ ID NO:5 or SEQ ID NO:6;
- c) said antibody is a monoclonal antibody; or
- d) said antibody is labeled.

35 17. A nucleic acid of Claim 13, wherein:

- a) said chemokine receptor is from a warm blooded animal selected from the group of birds and mammals, including a mouse or human;

68

- b) said nucleic acid comprises a sequence of Table 4;
- c) said nucleic acid is an expression vector; or
- d) said nucleic acid comprises a deoxyribonucleic acid nucleotide.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 96/05540

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C12N15/24 C12N1/21 C07K14/54 C07K1/36 C12P21/02
 //((C12N1/21,C12R1:19)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 6 C12N C07K C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J. BIOCHEM., vol. 106, 1989, pages 436-441, XP002028202 FURUTA R. ET AL.: "Production and characterization of recombinant human neutrophil chemotactic factor." cited in the application see the whole document, especially Materials and Methods	1-18, 21-28,34
A	---	19,20, 29-33
	-/-	

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *&* document member of the same patent family

2

Date of the actual completion of the international search
 25 March 1997

Date of mailing of the international search report

02.04.97

Name and mailing address of the ISA
 European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
 Fax (+ 31-70) 340-3016

Authorized officer

Mandl, B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 96/05549

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>PURIFICATION OF FERMENTATION PRODUCTS, EDS. LEROITH, SHILOACH AND LEAHY, ACS SYMPOSIUM SERIES, vol. 271, 1985, AMERICAN CHEMICAL SOCIETY, WASHINGTON, pages 21-49, XP002028203 RICKETTS R.T. ET AL.: "Application, sterilization and decontamination of ultrafiltration systems for large scale production." see the whole document, especially from p.31, last paragraph to p.37, first two paragraphs</p> <p>---</p>	1-18, 21-28,34
A	<p>WO 91 08483 A (BRIGHAM & WOMENS HOSPITAL) 13 June 1991 see page 9, line 32 - page 10, line 15 see page 18, line 4 - page 19, line 2</p> <p>---</p>	1-34
A	<p>JOURNAL OF EXPERIMENTAL MEDICINE, vol. 167, no. 4, 1988, pages 1364-1376, XP000121058 VAN DAMME J. ET AL.: "A novel NH₂-terminal sequence-characterized human monokine possessing neutrophil chemotactic, skin-reactive, and granulocytolysis-promoting activity." cited in the application page 1374, Summary</p> <p>---</p>	1-34
A	<p>US 4 599 176 A (WITTENBERGER UDO) 8 July 1986 see the whole document</p> <p>---</p>	1-34
A	<p>BIOTECHNOLOGY, vol. 3, 1985, pages 615-626, XP002028204 TUTUNJIAN R.S.: "Scale-up considerations for membrane processes." see the whole document</p> <p>-----</p>	1-34

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 96/05540

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9108483 A	13-06-91	AU 6747190 A		26-06-91
		EP 0502897 A		16-09-92
		US 5302384 A		12-04-94
-----	-----	-----	-----	-----
US 4599176 A	08-07-86	DE 3219248 A		24-11-83
		AT 391807 B		10-12-90
		BE 896807 A		16-09-83
		CH 663539 A		31-12-87
		EP 0095170 A		30-11-83
		FR 2527079 A		25-11-83
		GB 2130088 A, B		31-05-84
		JP 1007970 B		10-02-89
		JP 1697669 C		28-09-92
		JP 58210021 A		07-12-83
-----	-----	-----	-----	-----